FORMULATION AND EVALUATION OF TRANSDERMAL PATCH OF AQUEOUS EXTRACT OF *AZADIRACHTA INDICA* A.JUSS

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In partial fulfillment of the award of the degree of

MASTER OF PHARMACY

IN

BRANCH- I - PHARMACEUTICS

Submitted by

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This is to certify that the dissertation entitled **"FORMULATION AND EVALUATION OF TRANSDERMAL PATCH OF AQUEOUS EXTRACT OF *AZADIRACHTA INDICA* A. JUSS"** submitted by **Mr. T. SUTHEES [Reg. No: 261510203]** for the award of the degree of **"MASTER OF PHARMACY"** is a bonafide research work done by him in the Department of Pharmaceutics, Periyar College of Pharmaceutical Sciences, Tiruchirappalli under my guidance and direct supervision.

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ABBREVATIONS

WHO	World Health Organization
CAM	Complementary And Alternative Medicine
TM	Traditional Medicine
NDDS	Novel Drug Delivery System
TDDS	Transdermal Drug Delivery System
^{o}C	Degree Celsius
Cm^2	Square Centimeter
SC	Stratum Corneum
DMSO	Di Methyl Suphoxide
SLS	Sodium Lauryl Sulphate
e. g.	Example
Sq.ft	Square Feet
Rpm	Revolution Per Minute
Ml	Milliliter
Nm	Nanometer
mm	Millimeter
µg/ml	Micro gram per ml
IP	Indian pharmacopoeia
BP	British pharmacopoeia
Ph.Eur	European pharmacopoeia
m Pa s	One millipascal second

RH	Relative Humidity
Hrs	Hours
MU	Moisture Uptake
SD	Standard deviation
DW	Distilled water
AE	Aqueous extract
UV	Ultra violet spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
HPLC	High performance liquid chromatography

1. INTRODUCTION

1.1.TRADITIONAL MEDICINE SYSTEM

Traditional system of medicine is one of the centuries old practice and long-serving companion to humankind in the fight against disease and in leading a healthy life. Indigenous people have been using the unique approach of their traditional system of medicine for centuries and among the most renowned are the Chinese, Indian, African systems of medicine. Traditional medicine refers to any ancient and culturally based healthcare practice differing from scientific medicine and is largely transmitted orally by communities of different cultures.[1] The World Health Organization (WHO) observes that it is difficult to assign one definition to the broad range of characteristics and elements of traditional medicine, but that a working definition is essential. It thus concludes that the traditional medicines ‘‘[include] diverse health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness.[2]

Several developed countries have a major proportion of the population that uses traditional practice of health, especially medicinal plants, and have taken steps to preserve its popularity for historical and cultural reasons. Moreover, it has been reported that more than70% of the developing world’s population still depends on the complementary/ alternative systems of medicine, otherwise known as traditional medicine, for example, up to 80% of the population in Africa, 71% in Chile, and 40% in Colombia, and others.[3]

The modern health care service has posed immense threat to indigenous health practices because of their potential and speedy therapeutic effect. This has led to the disappearance and displacement of traditional systems of medicine. Also, traditional systems are undervalued by the people. However, the rise in population, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicine for a wide variety of human ailments.[1]

1.1.2. History of Traditional and Herbal Medicine

The use of plants as medicine goes back to the period of early humans. Fossil records human use of plants as medicines at least to the Middle Paleolithic age. Evidences of this early association have been found in the grave of a Neanderthal man buried 60,000 years ago. The earliest known medical document is a 4000-year-old Sumerian clay tablet that recorded plant remedies for various illnesses. By the time of the ancient Egyptian civilization, a great wealth of information already existed on medicinal plants. This information, along with the hundreds of other remedies, has been preserved in the Ebers papyrus for nearly 3500 years. [4]

The development of systematic pharmacopoeias dates back to 3000 BC, when the Chinese were already using more than 350 herbal remedies. China has demonstrated the best use of traditional medicine in providing health care. Ayurveda, a system of herbal medicines widely practiced in India, Sri Lanka, and Southeast Asia has more than 8000 plant remedies and uses nearly 35,000 to 70,000 plant species. Among the ancient civilizations, India have been known to be the richest repository of medicinal plants. About 8000 herbal remedies have been codified in Ayurveda. The Rigveda (5000 BC) has recorded 67 medicinal plants, the Yajurveda 81 species, the Atharvaveda (4500-2500 BC) 290 species, and the Charak Samhita (700 BC) and Sushrut Samhita (200 BC) have described properties and uses of 1100 and 1270 species, respectively, to compound the drugs and use.[5]

It is a well known fact that Traditional Systems of medicines always played important role in meeting the global health care needs. They are continuing to do so at present and shall play major role in future also. The system of medicines which are considered to be Indian in origin or the systems of medicine, which have come to India from outside and got assimilated in to Indian culture are known as Indian Systems of Medicine. India has the unique distinction of having six recognized systems of medicine in this category. They are: Ayurveda, Siddha, Unani and Yoga, Naturopathy and Homoeopathy.[6]

1.1.3. Traditional System of Medicines[7]

- Ayurveda
- Unani
- Homeopathy
- Siddha

1.1.4. Ayurveda

Ayurvedic medicine is a system of traditional medicine native to the Indian subcontinent and practiced in other parts of the world as a form of traditional medicine. In Sanskrit, the word ayurveda consist of the word sayus, means "longevity", and veda, means "related to knowledge" or "science". Evolving throughout its history, ayurveda remains an influential system of medicine in South Asia. The earliest literature on Indian medical practice appeared during the vedic period in India. The Susruta Samhta and the Charaka Samhta - where influential works on traditional medicine during this era. Ayurveda is considered to be a form of Complementary and Alternative Medicine (CAM) in the western world, where several of its methods, such as the use of herbs, massage, and yoga, are applied on their own as a form of CAM treatment.

Ayurveda is the ancient (before 2500 B.C.) Indian system of health care involving a holistic view of man, his health, and illness. Ayurvedic treatment of a disease consists of salubrious use of drugs, diets, and certain practices. Medicinal preparation is invariably complex mixtures, based mostly on plant products. Around 1, 250 plants are used in various ayurvedic preparations.

Many Indian medicinal plants have come under scientific scrutiny since the middle of the nineteenth century, although in a sporadic fashion.

The first significant contribution from ayurvedic material medica came with the isolation of the hypertensive alkaloids from the sarpagandha plant (*Rauwolfia serpentina*), valued in ayurveda for the treatment of hypertension, insomnia and insanity. This was the first important ancient-modern concordance in ayurvedic plants.

According to ayurveda, all objects in the universe including human body are composed of five basic elements (Panchamahabhutas) namely, earth, water, fire, air and vacum (ether).

There is a balanced condensation of these elements in different proportions to suit the needs and requirements of different structures and functions of the body matrix and its parts. The growth and development of the body matrix depends on its nutrition, i.e. on food. The food, in turn, is composed of the above five elements, which replenish or nourish the like elements of the body after the action of bio-fire (agni).

The tissues of the body are the structural whereas humours are physiological entities, derived from different combinations and permutations of Panchamahabhutas. Treatment of the disease consists in avoiding causative factors responsible for disequilibrium of the body matrix or of any of its constituent parts through the use of Panchkarma procedures, medicines, suitable diet, activity and regimen for restoring the balance and strengthening the body mechanisms to prevent or minimize future occurrence of the disease.

Use of these three measures is done in two ways. In one approach of treatment the three measures antagonize the disease by counteracting the etiological factors and various manifestations of the disease.

In the second approach the same three measures of medicine, diet and activity are targeted to exert effects similar to the etiological factors and manifestations of the disease process. These two types of therapeutic approaches are respectively known as Vipreeta and Vipreetarthkari treatments.

1.1.5. Siddha

Siddha system is one of the oldest systems of medicine in India. The term siddha means achievements and siddhars were saintly persons who achieved results in medicine. Eighteen Siddhars were said to have contributed towards the development of this medical system. Siddha literature is in tamil and it is practiced largely in tamil speaking part of India and abroad. The siddha system is largely therapeutic in nature.

This principles and doctrines of this system, both fundamental and applied, have a close similarity to ayurveda, with specialization in nitro-chemistry. According to this system the human body is the replica of the universe and so are the food and drugs irrespective of their origin.

Like ayurveda, this system believes that all objects in the universe including human body are composed of five basic elements namely, earth, water, fire, air and sky. The food, which the human body takes and the drugs it uses are all, made of these five elements. The proportion of the elements present in the drugs vary and their preponderance or otherwise is responsible for certain actions and therapeutic results. As in ayurveda, this system also considers the human body as a conglomeration of three humours, seven basic tissues and the waste products of the body such as feces, urine and sweat. The food is considered to be basic building material of human body which gets processed into humours, body tissues and waste products. The equilibrium of humours is considered as health and its disturbance or imbalance leads to disease or sickness.

This system also deals with the concept of salvation in life. The exponents of this system consider achievement of this state is possible by medicines and meditation.

The siddha system is capable of treating all types of disease other than emergency cases. In general, this system is effective in treating all types of skin problems particularly psoriasis, STD, urinary tract infections, diseases of liver and gastro intestinal tract, general debility, postpartum anemia, diarrhea and general fevers.

1.1.6. Unani

Unani system of medicines originated in Greece and is based on the teachings of Hippocrates and Gallen and it developed in to an elaborate medical system by Arabs, like Rhazes, Avicenna, Al-Zahravi, Ibne-Nafis and others. Unani medicines got enriched by imbibing what was best in the contemporary systems of traditional medicines in Egypt, Syria, Iraq, Persia, India, China and other Middle East countries. In India, unani system of medicine was introduced by Arabs and soon it took firm roots. The Delhi Sultans (rulers) provided patronage to the scholars of unani system and even enrolled some as state employees and court physicians. During 13th and 17th century A.D. Unani medicine had

its hey-day in India. During the British rule, unani system suffered a setback due to withdrawal of State Patronage, but continued to be practiced as the masses reposed faith in the system. It was mainly Sharif family in Delhi, the Azizi family in Lucknow and the Nizam of Hyderabad due to whose efforts unani medicine survived during the British period. In India, the concept of research in unani system of medicine was originally perceived by Masih-ul-Mulk Hakim Ajmal Khan in the 1920s. A versatile genius of his time, Hakim Ajmal Khan spotted Dr. Salim uzzaman Siddiqui- a chemist- for undertaking chemical studies on some important medicinal plants used in unani medicine.Dr. Siddiqui undertook the task visualized by Masih-ul-Mulk and his discovery of medicinal properties of a plant, commonly known as Asrol (*Pagalbooti*), led to sustained research that established the unique efficacy of this plant known all over the world as *Rauwolfia serpentina*, in neurovascular and nervous disorders, such as hypertension, insanity, schizophrenia, hysteria, insomnia and psychosomatic conditions, *etc*.,

1.1.7. Homeopathy

Homeopathy, founded by a German physician Samuel Hahnemann in 1790, is based on the idea that 'like cures like' that is substances that cause certain symptoms in a healthy person can also cure those same symptom in someone who is sick. This so called low of similar gives homeopathy its name 'homeo' for similar 'pathy' designating disease. In this experiment Hahnemann developed a method of 'potentizing' homeopathic remedies by diluting them in a water-alcohol solution and then vigorously shaking the mixtures.

The result convinced him that a high degree of dilution not only minimizes the side effects of the remedies but also simultaneously enhances their medical efficacy. Most Homeopathic remedies have undergone 'proving' or medical observation in which healthy individuals are given doses of undiluted homeopathic substances.

Mental, emotional, and other details of the patients are most important. This leads the physician to a better understanding of which remedy will best suits a particular set of symptoms. Over the past 200 years, providing for almost 2,000 substances have been conducted.

1.1.8. Current Status of Herbal Medicine

Currently more than 80% of the world population depends on traditional and plant derived medicine because, plants are important sources of medicines and presently about 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient. In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources.[8]

1.1.9. Future importance of Herbal Medicine

It is estimated that there are about 350,000 species of existing plants (including seed plants, bryophytes, and ferns), among which 287,655 species have been identified as of 2004. Relatively small percentages (1 to 10%) of these are used as foods by both humans and other animal species. It is possible that even more are used for medicinal purpose .[8]

The strategy has four main objectives, in line with those of WHO's medicines strategy:

Objectives

- To integrate relevant aspects of traditional medicine within national health care systems by framing national traditional medicine Policies and implementing programmes
- To Promote the safety, efficacy and quality of Traditional Medicine Practices by providing guidance on regulatory and quality assurance standards
- To increase access to, and affordability of, traditional medicine
- To promote rational use of traditional medicine implementation

1.1.10. Policy

A national policy is urgently needed in those countries where traditional medicine is popularly used in primary health care, and governments are becoming increasingly aware of it. For instance, in the Western Pacific Region, only four countries had a national policy on traditional medicine in 1994 the number had risen to 14 by 2001.

In general, such policy should include a definition of the government's role in developing traditional medicine in the health-care delivery system, and contain a mission as well as goals and objectives. Integration of traditional medicine into the national health system will enable the two systems to work effectively together, to the benefit of the government, patients and consumers.

1.1.11. Safety, efficacy and quality

- Governments need to undertake a series of activities to ensure the safety and efficacy of traditional medicine, including establishment of a national expert committee, formulation of national regulations for herbal medicines, licensing of the practice of traditional medicine, and provision of support for research
- Member States are becoming increasingly aware of the importance of the safety and efficacy of traditional medicine. Countries with regulations on herbal medicines have increased from 50 in 1994 to 70 in 2001. National research institutes for traditional medicine have also been established and research funding has increased. For example, in the African Region, 21 out of 46 countries have institutes carrying out research in traditional medicine

Access

Low-income countries need inexpensive and effective treatment for common diseases. The fact that traditional medicine practitioners live and work at community level makes such treatment available and affordable to most of the population. The role of traditional practitioners should be recognized and cooperation between them and community health-workers should be strengthened

Rational use

Traditional medicine is provided not only by traditional practitioners, but also by medical doctors. In Canada, 57% of herbal therapies, 31% of chiropractic and 24% of acupuncture treatment are provided by general practitioners. In the Netherlands, 50% of general practitioners prescribe herbal medicines and provide manual therapies and acupuncture.

The communication between doctors and traditional practitioners should be strengthened and appropriate training programmes established. Further, as traditional medicine is mostly used as self-care, health authorities should develop education and training programmes for consumers on its proper use.[9]

1.1.12. NEEM

The Neem tree **(*Azadirachta indica*)** is a tropical evergreen tree native to India and is also found in other southeast countries. In India, neem is known as " the village pharmacy" because of its healing versality and it has been used in Ayurvedic medicine for more than 4,000 years due to its medicinal properties. Neem is also called "arista" in Sanskrit – a word that means Perfect, complete and imperishable.

The seeds, bark and leaves contain compounds with proven antiseptic, antiviral, antipyretic, anti- inflammatory, anti-ulcer and antifungal uses. The Sanskrit name "nimba" comes from the term "nimbitisyasthyamdadati" which means 'to give good health'**.**

Important uses of various parts of neem tree

- ✓ **Neem oil** is extracted from the seeds of the neem tree and has insecticidal and medicinal properties due to which has been used for thousands of years in pest control, cosmetics, medicines, etc.,
- ✓ **Neem seed cake (residue of need seeds after oil extraction)** when used for soil amendment or added to soil, not only enriches the soil with organic matter but also lowers nitrogen losses by inhibiting nitrification. It also works as a nematicide
- ✓ **Neem leaves** are used to treat chickenpox and warts by directly applying to the skin in a paste form or by bathing in water with neem leaves. In order to increase immunity of the body, neem leaves are also taken internally in the form of neem capsules or made into a tea. The tea is traditionally taken into internally to reduce fever caused by malaria

- ✓ **In Ayurveda,** neem leaves are used in curing neuromuscular pains. Neem leaves are also used in storage of grains
- ✓ **Twigs of neem** are also used in India and Africa as tooth brushes. Nowadays tooth pastes with neem extracts are also available commercially
- ✓ **Neem (leaf and seed)** extracts have been found to be spermicidal and thus research is being conducted to use neem extracts for making contraceptives
- ✓ **Neem** produces pain relieving, anti-inflammatory and fever reducing compounds that can aid in the healing of cuts, burns, ear aches, sprains and headaches, as well as fevers
- ✓ **Neem bark and roots** also have medicinal properties. Bark and roots in powdered form are also used to control fleas and ticks on pets
- ✓ **Neem** has **anti-bacterial properties** that helps in fighting against skin infections such as acne, psoriasis, scabies, eczema, etc.,
- ✓ **Neem extracts** also help sin treating diabetes, aids, cancer, heart disease, herpes, allergies, ulcers, allergies, ulcers, hepatitis and several other disease
- ✓ **Neem leaves and neem extracts** are used to manufacture health and beauty care products[10]

1.1.13. NEEM IN TRADITIONAL SYSTEM OF MEDICINE

The therapeutic efficacy of neem must have been known to man since antiquity as a result of constant experimentation with nature. Ancient man observed the unique features of this tree a bitter taste, non- poisonous to man, but deleterious to lower forms of life.

Ayurveda

The word neem is derived from Sanskrit Nimba, which means “to bestow health”, the various Sanskrit synonyms of neem signify the pharmacological and therapeutic effect of the tree. Ayurveda the word neem is derived from Sanskrit Nimba, which means “to bestow health”, the various Sanskrit synonyms of neem signify the pharmacological and therapeutic effects of the tree.It has been nicknamed Neta- a leader of medicinal plants, Pichumarda- anti leprotic, Ravisambba- sunray-like effects in providing health,

Arishta - resistant to insects, beetal-cooling (cools the human system by giving relief in disease caused by hotness, such as skin disease and fever) and Krimighana - Anthelmintic.

Ayurvedic products

Some of the important poly herbal neem preparations of Ayurvedic pharmacopoeia and their main uses are :

- Aparjith Dhttp - fumigant for purification for air (air sterilizer)
- Erhamanisthadith Kwath - skin disease
- Dhatturtailam - oil for skin disease and muscular pain
- Jatyaditailam - oil for ulcer
- Jeevantiadi kashyam - for small pox
- Laghumanjishtadi Kwath - decoction for skin disease
- Kandavadu Lepah - poultice for itching
- Mahatikatamghritam - butter fat for skin diseases
- Nimbadikashyam - for skin disease [11]

Unani

In the Greco - Persian system of medicine (Unani tibb), which was patronized by muslim rulers in the medieval era in the Indian subcontinent, the leaves and fruit were in the pharmacopeia. As per this system, neem is cold 1°,dry 2°, a resolvent and blood purifier. Neem leaves, called "burgh-i-neem", are said to expel foul wind from the body and heal ulcers in the urinary passage it is an emenagogue and good for skin disease.

Unani Products

Neem leaves, bark, seed and oil are incorporated in some of the unani preparations as follow:

- ArqGaz - a distillate from all five parts of the neem tree, used for fevers due to inflammation of the spleen
- Arqharabhara - a distillate from the seed coat, tonic for the lungs
- Arqmurakkabmussaffakhun - a distillate from the seed coat, a tonic for the lungs

- Habnarkachur - anti-inflammatory for children
- Habsiyahchatham - for application inside the eyelid in conjunctivitis[12]

Homeopathy

Homeopathic remedy *Azadirachta indica* to treat health problems. Neem used in homeopathy system of medicine due to its immense health benefiting qualities. It is boon for skin when used both internally and externally. It detoxifies blood and cures acne, pimples, boils and similar condition neem is bitter in taste. But this bitterness is responsible for its therapeutic efficacy of this tree.

Homeopathic remedy

- **Ozena:** A chronic disease of the nose accompanied by a field discharge. It is characterized by structural changes in nose
- **Pemphigus:** It is a skin disease in which there are watery blisters
- **Scabies**: It is disease which is spread from one person to another very easily and characterized by itching and small raised red spots. It is caused by itch mite
- **Leprosy:** It is a disease that causes symptoms on skin, mucous membrane, and nerves. It causes loss of color of skin and in severe cases causes disfigurement and deformities
- **Helminthiasis:** It is a disease which is caused by worms
- **Gonorrhea**: It is sexually transmitted disease which causes discharge from urethra or vagina
- **Glossitis:** Inflammation of the tongue
- **Ophthalmia:** Inflammation of the eye
- **Asthma:** It is a respiratory condition which is marked by attacks of spasm in lungs, causing difficulty in breathing. It is basically allergic reaction
- **Catarrh:** It is basically inflammation of the mucous membrane which causes excessive discharge of mucus in the nose or throat
- **Constipation:** It is a condition which characterized by hardened stools
- **Uterine weakness:** it is associated with pregnancy, childbirth, lifting, coughing, damage to or weakness of the muscles[13]

Siddha

Siddha medicine is one of the important traditional medicinal systems, which is popular among Tamils. Siddha mediccines have effective treatment for many skin problems such as psoriasis, eczema, leucoderma, skin rashes, pimples, itching, acne, wrinkles, black spots, white spots and breast nipple discoloration. This safe and cost effective natural treatment modality is also good for skin allergy, skin whitening and other skin related problems.

Siddha Medicinal Uses

- The leaf juice in the dose of 10-20 ml is administered along with equal amount of honey three times a day for jaundice, intestinal worms etc.,
- Neem oil along with equal amount of castor oil is given early morning in empty stomach to remove the intestinal worms
- The leaves of this plant along with turmeric is ground together and applied over the areas of insect bite, sori, (skin disease), sirangu (scabies), fungal infection, karappan (eczema)
- The decoction prepared from leaves and bark is used to clean the ulcers and skin disease
- The leaf paste is applied over the burns
- The decoction prepared from the flowers is helpful in treating Gunmam or abdominal disorders
- The fruit is dried and the seeds are removed, this is then powered and is given in the dose of 1-10 grams two times a day for periodic fever
- The oil prepared from neem is an anti-septic and applied externally for ulcers, eczema, and sirangu
- Neem oil is used over herpes infection keelvayu or arthritis and kandamalai (cervical lymphadenitis)
- The neem oil , 5-6 drops is given along with milk in diabetes Tender leaves of neem is an excellent medicine for diabetic patients [14].

1.2. NOVEL DRUG DELIVERY SYSTEM [15]

The aim of Novel Drug Delivery System is to provide a therapeutic amount of drug to the appropriate site in the body to accomplish promptly and then maintain the desired drug concentration. The drug- delivery system should deliver drug at a rate control by the necessarily of the body over a specified term of treatment. These idealized objective switches to the two aspects most important to drug delivery are as follows:

I. Spatial Drug Delivery:

Targeting a drug to a particular organ or tissue.

II. Temporal Drug Delivery:

The drug delivery rate to the target tissue is controlled.

The prime areas of research and development for NDDS are:

- Liposomes
- Niosomes
- Nanoparticles
- Transdermal drug delivery
- Implants
- Oral system
- Micro encapsulation / Microcapsules
- Polymer in drug delivery

Novel drug delivery system can be divided into classes:

1. Sustained diffusion drug delivery system
2. Controlled diffusion drug delivery system

Sustained diffusion drug delivery system

It is a pharmaceutical dosage from formulated to retard the diffusion of a therapeutic effect such that its look in the systemic circulation is delayed and or prolonged and the plasma profile is sustained in duration. The onset of its pharmaceutical action is often slow, and the duration of its therapeutic effect is sustained. (Eg: coated granules)

Controlled diffusion drug delivery system

This system has a meaning that goes beyond the scope of sustained drug action. It Many fests a predictability and reproducibility in the drug diffusion kinetics. The diffusion of drug substances from a controlled diffusion drug delivery system gains at a rate profile that is not only predictable kinetically but also reproduced from one unit to another.

They are classified as follows:

I. Rate - preprogrammed drug delivery system

II. Activation - Modulated drug delivery system

III. Feed - Back Regulated drug delivery system

IV. Site - Targeting drug delivery system

1.2.1. Merits of drug delivery system

1. Better treatment of many chronic illnesses. Eg. Cancer, Asthma, Arthritis
2. Increased Bio- availability
3. Reduction in the occurrence and badness of untoward systemic side effects related to high blood plasma drug concentration
4. Sustenance of the total amount of drug administered over the period dose periods
5. Reduction in the total amount drug administered over the period of drug treatment which reduce occurrence of systemic and local side effects
6. Prevention from first pass metabolism and gastrointestinal tract degradation
7. Better patient compliance effect from the reduction in the number and frequency of doses needed to maintain the want therapeutic responses
8. Targeting the drug molecule towards the affected tissue or organ make smaller the toxicity to the normal tissues
9. Versatile and pH dependent system diffusion the drug whenever the body demands
10. Biocompatibility

1.2.2. Limitations

Factors that limit its usage

1. Physiological factors such as gastro intestinal enzyme, activates pH /gastric and intestinal transit rates, food and disease which often influence drug bioavailability from conventional dosage forms may interfere with the accuracy of control diffusion and absorption of drug from the system.
2. The products which remain intact may become accommodates at some sites results slow diffusion of drug from the dosage form may produce a high localized concentration of drug which produces local irritation.
3. Drugs with half - life of 1hr or less are difficult to be formulated as sustained diffusion formulation. The high rate of elimination of such drugs from the body requires an highly large maintenance dose which provides 8-12 hrs of continuous diffusion.
4. Since these products contain a large amount of drug. There is a chance of unsafe over dosage, if the product is improperly made and the total drug contained there is diffusion at one time or over too short time of interval.
5. It is difficult to cease the therapy once after administration may be for reasons of toxicity or any other.
6. It may be not suitable to encompass potent drugs in such system.

1.3. TOPICAL DRUG DELIVERY SYSTEM [16]

Topical drug delivery can be defined as application of drug via skin to directly treat or cure the skin disorders. These systems are generally used for local skin infection like fungal infection or in place where other routes of the drug administration fails. Topical dosage forms are generally confined to a small area anywhere in the body through ophthalmic, rectal, vaginal and skin as route. Skin is one of the most easily accessible organ of human body.

Skin of an average adult body covers a surface of about $2m^2$ and receives around one-third of the blood circulating through the body. Over the past three decades, controlled drug delivery has become increasingly important in the pharmaceutical industry. The surface of human skin is known to contain, on an average, 10 -70 hair follicles and 200 to 250 sweat ducts on every cm2 of the skin area. Skin is a very difficult barrier to the ingress materials allowing only small quantities of drug molecules to penetrate over a period of time.

Transport of hydrophilic or charged molecules is especially difficult attributable to the lipid-rich nature of the *Stratum corneum* and its low water content this layer is composed of about 40% lipids, 40% protein, and only 20% water. Transport of lipophilic drug molecules is facilitated by their dissolution into intercellular lipids around the cells of the *Stratum corneum*. Absorption of hydrophilic molecules into skin can occur through 'pores' or openings of the hair follicles and sebaceous glands, but the relative surface area of these openings is barely 1% of the total skin surface. This small surface area limits the amount of drug absorption.

Precutaneous absorption of drug molecules is a key factor of particular importance in the case of topical drug delivery systems because the drug has to be absorbed to an adequate extent and rate to achieve and maintain uniform, systemic, therapeutic levels throughout the duration of use.

In general, once drug molecules cross the stratum corneal barrier, passage into deeper dermal layers and systemic uptake occurs relatively quickly and easily. Drugs with the lipophillic character, are better suited for topical delivery.

These systems ensure that the drug get into the body and reach the area where it is needed. These preparations are applied onto the skin surface for providing local or systemic effects. Topical route favours safe and effective delivery of drug molecules with lower doses as compared to the conventional system. Drugs via skin reaches the desired area in optimum concentration, dropping the chances of side effects leading to increased bioavailability and increased patient compliance. Dermatological conditions i.e. skin disease affects the population and has been observed as one of the top 15 medical conditions for which prevalence and healthcare spending have increased in the last decade.

Advancements in the life sciences united with a growing market for dermatological have facilitated the emergence of better topical formulations and drug delivery systems. The present and emerging approaches of optimizing the topical dermatological agents delivery (i.e small and large molecules) includes the use of chemical enhancers, liposomes, bio-polymers (sodium hyaluronate), particulate carriers (microspheres and lipid nanoparticles), occlusion (via dressings and patches) topical peels, topical sprays and foams, temperature (heat), iontophoresis and ultrasound.

These delivery approaches are a significant advancement over conventional systems (i.e creams, lotions, ointments and pastes) and are likely to enhance efficacy and tolerability, improve patient compliance (which include dermatology life quality), and also fulfill other required needs of the topical dermatological market.

However, the limited dermal and transdermal delivery of many small and large molecules is a significant challenge because of the unyielding barrier properties of the skin. This paper reviews the application of a novel topical delivery system, employing sophisticated carriers built from nanoscale components, to the delivery of several therapeutic agents and discusses progress toward its clinical application.

1.3.1. Advantages of topical drug delivery systems [17]

- Avoidance of the first pass metabolism
- Convenient and easy to apply
- Avoidance of risks and inconveniences of the intravenous therapy and of diverse conditions of absorption like pH changes, presence of enzymes, gastric emptying time
- Easily terminate the medications, when needed
- Deliver drug more selectively to a specific site
- Avoidance of the gastro-intestinal incompatibility
- Providing utilization of drugs with short biological half life, narrow therapeutic window
- Improved patient compliance
- Provide suitability for self-medication
- Achievement of effectiveness with lower total daily dose of drug by continuous drug input
- Avoids fluctuation in drug levels, inter- and intra patient variations
- A quite large area of application in comparison with buccal or nasal cavity
- Ability to deliver drugs more selectively to a specific site

1.3.2 Disadvantages of topical drug delivery systems

- Skin irritation or dermatitis may occur due to the drug or excipients
- Poor permeability of some drugs through skin
- Drugs with larger particle size can't be easily absorbed through the skin
- Risk of allergenic reactions
- Can be used only for the drugs which need very small plasma concentration for action

1.4. SKIN [18]

The skin completely covers the body and is continues with the membranes lining the body orifice.

- It protects the underlying structures from injury and from invasion by microbes
- It contains sensory (somatic)nerve endings of pain, temperature and touch
- It is involved in the regulation of body temperature

1.4.1. Structure of the skin

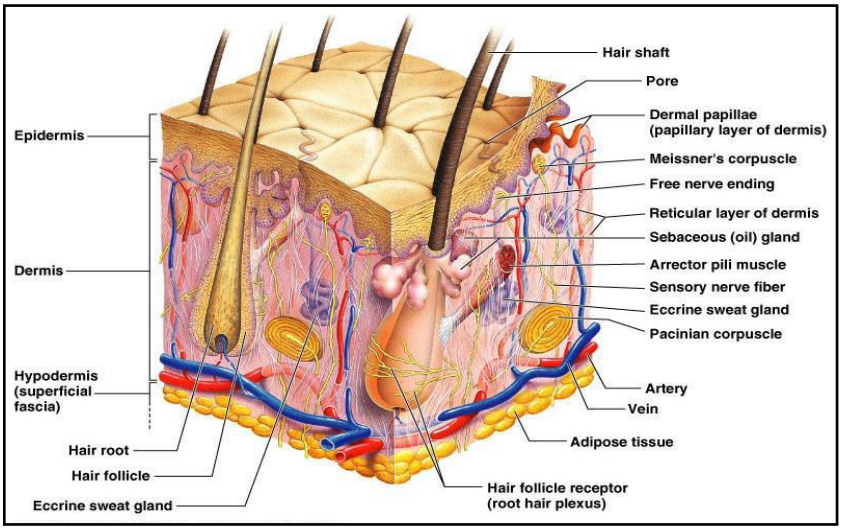


Fig No.1: Structure of skin

The skin is the largest organ of human body, accounting for about 15% of the whole adult body weight. Skin is one of the most readily accessible parts of the human body for topical administration. Penetration of molecules in the skin mainly occurs through three routes through intact stratum corneum, through the sebaceous follicle and through sweat ducts.

Topical drug delivery approach is used for localized action on the body through skin, ophthalmic, rectal and vaginal as topical routes.

Skin performs various important functions:

- Protection against the physical, biological and chemical assailants
- Prevention of excess loss of water from the body
- Vital role in the thermoregulation
- Enzyme in epidermis can denature the drugs

The skin consists of three layers that are the epidermis, the dermis and the subcutaneous tissue. An average human skin surface contain, on an average 40-70 hair follicles and 200-300 sweat ducts on every cm^2 of the skin. The pH of the skin vary from 4- 5.6 the skin of an average adult body covers a surface area of about $2m^2$ and receives about one third part of the blood circulating through the body.

1.4.2. Epidermis

It is a stratified squamous epithelium layer which is composed primarily of two types of cells dendritic and keratimocytes cells. The epidermis layer harbour a number of other cells such as melanocytes, Merkel cells and Langerhans cells. But the keratinocytes cells type comprises the majority of the cells by far.

- ✓ ***Stratum germinativum*** (basal layer or rowing layer): It contains column-shaped keratinocytes that attach to the zone of basement membrane with their long-axis perpendicular to the dermis
- ✓ ***Stratum spinosum*** (prickly cell layer or squamous cell layer): Its thickness vary from 5-10 cells. Intercellular spaces between spinous cells are bridged by abundant desmosomes (adhering spot) to promote coupling between cells of the epidermis and provide resistance to the physical stresses
- ✓ ***Stratum granulosum*** (granular layer): It consists of living cells, these are responsible for further synthesis and modification of the proteins involved in keratinization. It is 1-3 cells layer in thickness
- ✓ ***Stratum corneum*** (horny layer): the corneocytes are rich in protein and low in lipid content (hydrophilic nature) are surrounded by a continuous extra cellular lipid matrix
- ✓ **Malpighian layer** (pigment layer): the layer whose protoplasm has not yet change into horny material

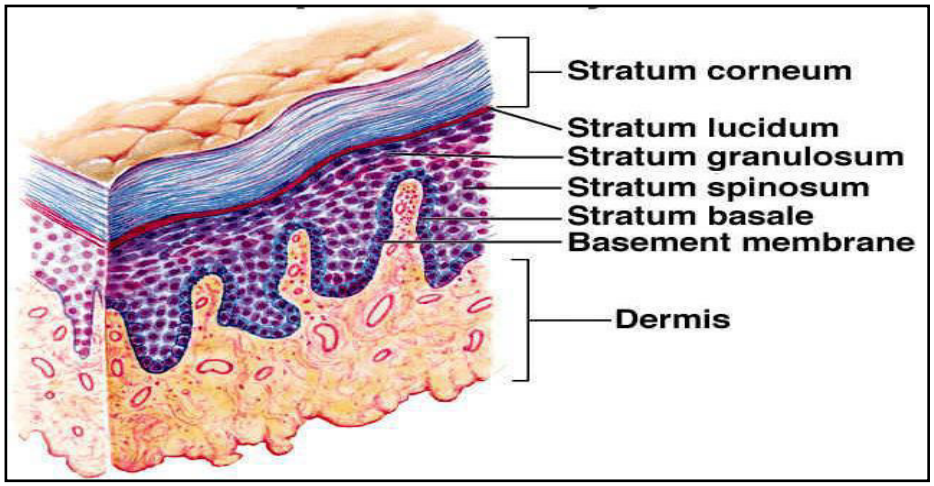


Fig No.2: Structure of skin layers

1.4.3. Dermis

It lies beneath the epidermis 1.5 - 4 mm thick (thickest of the three layers of the skin). It is like home for most of the skin's structures including sweat glands and oil glands, hair follicles, nerve endings, and blood and the lymph vessels. The main components of the dermis are collagen and elastin. It stores much of the body's water supply.

The dermis also contains the scavenger cells from the immune system. In an event that a foreign organism tries to pass through epidermis, these cells will engulf and destroy.

It is an integrated system of fibrous, amorphous and filamentous connective tissue that accommodates stimulus induced entrance by nerve, vascular-networks, fibroblasts, appendages, mast cells. Its thickness ranges from 2000-3000µm. The principal component of the dermis is collagen and it represents 70% of the skin's dry weight

1.4.4. Subcutaneous tissue (Connective Tissue)

The subcutaneous tissue or hypodermis is not actually considered as a true part of the structured connective tissue, which comprises of loose textured, fibrous, white, connective tissue containing blood and lymph vessels, secretary pores of the sweat gland and the cutaneous nerves. Most investigators consider that drug permeating through the skin enters the circulatory system before reaching the hypodermis, although the fatty tissue could serve as a depot of the drug.

1.4.5.Blood and lymph vessels

Arterioles form a fine network with capillary branches supplying sweat glands, sebaceous glands, hair follicles, and the dermis. Lymph vessels form a network through the dermis.

1.4.6. Sensory nerve endings

Sensory receptors (specialized nerve endings) sensitive to touch, temperature, pressure, and pain are widely distributed in the dermis. Incoming stimuli activate different types of sensory receptors. The pacinian corpuscle is sensitive to deep pressure. The skin is an important sensory organ through which individuals receive information about their environment. Nerve impulses, generated in the sensory receptors in the dermis, then to the sensory area of the cerebrum where the sensations are perceived.

1.4.7. Sweat glands

These are widely distributed throughout the skin and are most numerous in the palms of the hands, soles of the fet, axillae, and groins. They are formed from epithelial cells. There are two types of sweat gland. The commonest type opens onto the skin surface through tiny pores, and the sweat produced here is clear, watery fluid important in regulating body temperature.

1.5. Functions of the skin [19]

1.5.1. Protection

The skin forms relatively water proof layer, provided mainly by its epithelium, which protects the deeper and more delicate structures. As an important non-specific defence mechanism its act as a barrier against:

- Invasion by micro organism
- Chemicals
- Physical agents
- dehydration

The epidermis contains specialized immune cells called langerhans cells, which are a type of microphage. Due to the presence of the sensory nerve endings in the skin the body reacts by reflex action to unpleasant or painful stimuli, protecting it from further injury.

1.5.2. Regulation of body temperature

Body temperature remains fairly constant at about 36.8℃ across a wide range of environmental temperature ensuring that the optimal range for enzyme activity required for metabolism is maintained. In health, variation are usually limited to between 0.5 and 0.75℃, although it rises slightly in the evening, during exercise and in women just after ovulation

1.5.3. Heat production

When metabolic rate increases, body temperature rises, and when it decrease body temperature falls. Some of the energy diffusion during metabolic activity is in the form of heat and the most active organs produced in the body and heat lost to the environment. The principal organs involved are

- Skeletal muscles- contraction of skeletal muscles produces a large amount of heat and the more strenuous the muscular exercise, the greater the heat produced
- The liver is very metabolically active and heat is produced as a by-product. Metabolic rate and heat production are increased after eating
- The digestive organs produce heat during peristalsis and during the chemical reactions involved in digestion

1.5.4. Heat loss

Most heat loss from the body occurs through the skin. Small amounts are lost in expired air, urine, and faeces. Only heat loss through the skin can be regulated. Heat loss by the outer routes cannot be controlled.

Heat loss through the skin is affected by the difference between body and environmental temperatures, the amount of the body surface exposed and the type of clothes worn. Air insulates against heat loss when trapped in layers of clothing and between the skin and clothing.

For this reason several layers of light weight clothes provide more effective insulation against low environmental temperatures than one heavy garment.

1.5.5. Drug transport across skin

There are mainly two important layers in the skin epidermis and dermis. Blood vessels are profusely distributed beneath the skin in the subcutaneous layer.

There are two primary mechanisms intended for drug absorption through the skin they are:

- Intercellular
- Trans cellular

The another most common route of delivery is through the pilosebaceous route. Permeation tend to occur through intercellular matrix, but via trans cellular pathway it has been shown to provide a quicker alternative route for highly polar molecules.

In normal intact skin it is considered that the keratinized corneocytes and the large non-polar lipid intercellular cement of the horny layer are the major factors involved in maintenance of efficient barrier for drugs. The drug penetration for skin can be improved by using organic solvents such as propylene glycol, Di Methyl Shulphoxide (DMSO) and surfactants

The permeation enhancers alters the barrier properties of the *stratum corneum* by type of mechanism which includes enhancing solubility, partitioning the *stratum corneum* and fluidizing the crystalline structure of the *stratum corneum*

New technologies now allow other drugs to be absorbed via skin. These can be used to treat not just the affected areas of the skin but the whole body by systemic route. The barrier resides in the outmost layer of the epidermis and the *stratum corneum*, as evidenced by just about equal rates of penetration of chemicals through the isolated *stratum corneum* or whole skin.

1.6. TRANSDERMAL DRUG DELIVERY SYSTEM

The (TDDS) are defined as self-contained, discrete dosage forms which, when applied to the intact skin, deliver the drug(s), through the skin, at a controlled rate to the systemic circulation. Transdermal drug delivery is a viable administration route for potent, low-molecular weight therapeutic agents which cannot withstand the hostile environment of gastrointestinal tract and/or subject to considerable first-pass metabolism by the liver.

Fig No.3:Transdermal patch

Transdermal drug delivery systems are topically administered medicaments in the form of patches that deliver drugs for systemic effects at a predetermined and controlled rate. A transdermal drug delivery device, which may be of an active or a passive design, is a device which provides an alternative route for administering medication. These devices allow for pharmaceuticals to be delivered across the skin barrier.

In theory, transdermal patches work very simply. A drug is applied in a relatively high dosage to the inside of a patch, which is worn on the skin for an extended period of time. Through a diffusion process, the drug enters the bloodstream directly through the skin. Since, there is high concentration on the patch and low concentration in the blood, the drug will keep diffusing into the blood for a long period of time, maintaining the constant concentration of drug in the blood flow [20].

1.6.1. Advantages of TDDS [21]

This approach to drug delivery offers many advantages over traditional methods:

- As a substitute for the oral route
- Transdermal drug delivery enables the avoidance of gastrointestinal absorption, with its associated pitfalls of enzymatic and pH associated deactivation
- This method also allows for reduced pharmacological dosaging due to the shortened metabolization pathway of the transdermal route versus the gastrointestinal pathway
- The patch also permits constant dosing rather than the peaks and valleys in medication level associated with orally administered medications. Multi-day therapy with a single application. Rapid notification of medication in the event of emergency, as well as the capacity to terminate drug effects rapidly via patch removal

1.6.2. Disadvantages of TDDS

- The drug that requires high blood levels cannot be administered and may even cause irritation or sensitization of the skin
- The adhesives may not adhere well to all types of skin and may be uncomfortable to wear
- High cost of the product is also a major drawback for the wide acceptance of this product
- Properties that influence transdermal delivery diffusion of the medicament from the vehicle
- Penetration through the skin barrier activation of the pharmacological response

1.6.3. Pathway of Transdermal Permeation

The permeation of drugs through the skin includes the diffusion through the intact epidermis and through the skin appendages, *ie.,* hair follicles and sweat glands, which form shunt pathways through the intact epidermis. However, these skin appendages occupy only 0.1% of the total human skin surface and the contribution of this pathway is usually considered to be small (with only a few exceptions having been noted). As stated above, drug permeation through the skin is usually limited by the *Stratum corneum*. Two pathways through the intact barrier may be identified (Fig No.4) the intercellular lipid route between the corneocytes and the transcellular route crossing through the corneocytes and the intervening lipids that is, in both cases the permeant must diffuse at some point through the intercellular lipid matrix, which is now recognized as the major determinate of percutaneous transport rate[22].

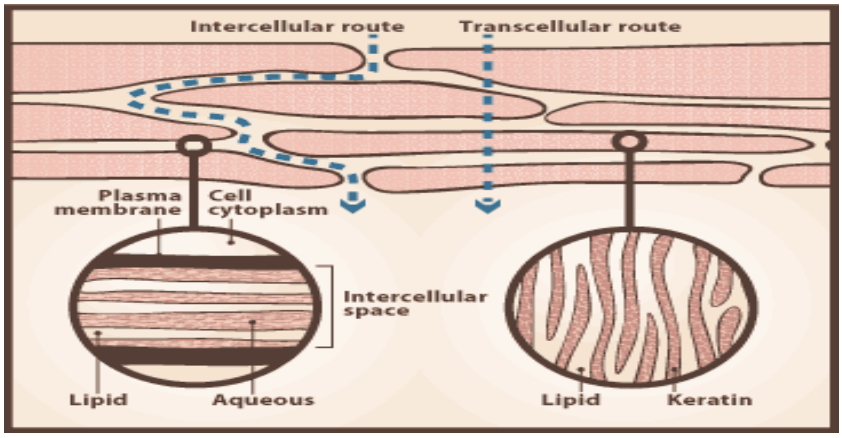


Fig No.4: Drug Pathway Of Skin

1.6.4 Kinetics of Transdermal Permeation

Knowledge of skin permeation kinetics is vital to the successful development of transdermal therapeutic systems. Transdermal permeation of a drug involves the following steps:

1. Sorption by *stratum corneum*.

2. Penetration of drug through epidermis.

3. Uptake of the drug by the capillary network in the dermal papillary layer.

This permeation can be possible only if the drug possesses certain physiochemical properties. The rate of permeation across the skin is given by

$$dQ/dt = Ps\ (Cd - Cr)\text{----}1$$

Where Cd and Cr are the concentration of the skin penetrant in the donor compartment i.e. on the surface of stratum corneum and in the receptor compartment i.e. body respectively. Ps is the overall permeability coefficient of the skin tissue to the penetrant. This permeability coefficient is given by the relationship

$$Ps = D_{ss}K_s/h_s\text{---------}2$$

Where Ks is the partition coefficient for the interfacial partitioning of the penetrant molecule from a solution medium or a transdermal therapeutic system on to the stratum corneum, Dss is the apparent diffusivity for the steady state diffusion of the penetrant molecule through a thickness of skin tissues and hs is the overall thickness of skin tissues. As Ks, Dss and hs are constant under given conditions the permeability coefficient Ps for a skin penetrant can be considered to be constant. From equation (1) it is clear that a constant rate of drug permeation can be obtained only when Cd >> Cr i.e. the drug concentration at the surface of the stratum corneum Cd is consistently and substantially greater than the drug concentration in the body Cr.

The equation becomes

$$dQ/dt = P_sC_d\text{----------}3$$

The rate of skin permeation is constant provided the magnitude of Cd remains fairly constant throughout the course of skin permeation. For keeping Cd constant the drug should be diffusiond from the device at a rate Rr i.e. either constant or greater than the rate of skin uptake Ra i.e. Rr>> Ra. Since Rr>> Ra , the drug concentration on the skin surface Cd is maintained at a level equal to or greater than the equilibrium solubility of the drug in the stratum corneum Cs .i.e. Cd>>Cs.

Therefore a maximum rate of skin permeation is obtained and is given by the equation:

$$(dQ/dt)_m = P_sC_s\text{---------}4$$

From the above equation it can be seen that the maximum rate of skin permeation depends upon the skin permeability coefficient Ps and is equilibrium solubility in the stratum corneum Cs. Thus skin permeation appears to be *stratumcorneum* limited [23].

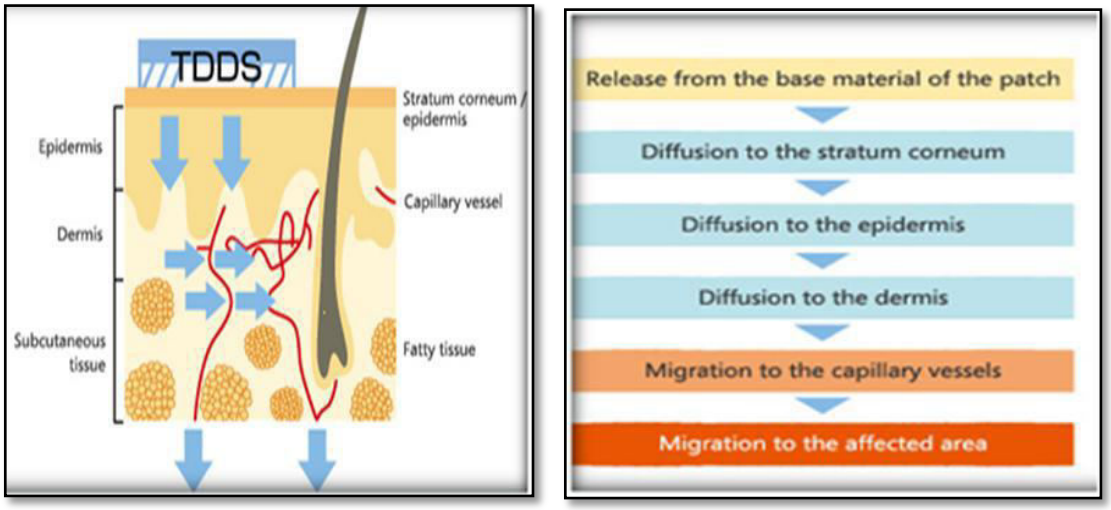


Fig No.5: Skin permeation

1.6.5. Basic Components of Transdermal Drug Delivery Systems

1. Polymer matrix or matrices
2. The drug
3. Permeation enhancers
4. Other excipients

1. Polymer Matrix

The Polymer controls the diffusion of the drug from the device. Possible useful polymers for transdermal devices are:

a. Natural Polymers: e.g., cellulose derivatives, Zein, Gelatin, Shellac, Waxes, Proteins, Gums and their derivatives, Natural rubber, Starch etc.

b. Synthetic Elastomers: e.g., polybutadieine, Hydrin rubber, Polysiloxane, Silicone rubber, Nitrile, Acrylonitrile, Butyl rubber, Styrenebutadieine rubber, Neoprene etc.

c. Synthetic Polymers: e.g., polyvinyl alcohol, Polyvinyl chloride, Polyethylene, Polypropylene, Polyacrylate,Polyamide ,Polyurea, Polyvinyl pyrrolidone, Polymethyl methacrylate, Epoxy etc.

2. Drug

For successfully developing a transdermal drug delivery system, the drug should be chosen with great care. The following are some of the desirable properties of a drug for transdermal delivery.

Physicochemical properties

- The drug should have a molecular weight less than approximately 1000 Daltons
- The drug should have affinity for both lipophilic and hydrophilic phases. Extreme partitioning characteristics are not conducive to successful drug delivery via the skin
- The drug should have low melting point
- Along with these properties the drug should be potent, having short half life and be non- irritating

3. Permeation Enhancers

These are compounds which promote skin permeability by altering the skin as a barrier to the flux of a desired penetrant. These may conveniently be classified under the following main headings:

A. Solvents

These compounds increase penetration possibly by swallowing the polar pathway and/or by fluidizing lipids. Examples include water alcohols – methanol and ethanol; alkyl methyl sulfoxides – dimethyl sulfoxide, alkyl homologs of methyl sulfoxide dimethyl acetamide and dimethyl formamide; pyrrolid- ones- 2 pyrrolidone, N-methyl, 2-purrolidone; laurocapram (Azone), miscellaneous solvents- propylene glycol, glycerol, silicone fluids, isopropyl palmitate.

B. Surfactants

These compounds are proposed to enhance polar pathway transport, especially of hydrophilic drugs. The ability of a surfactant to alter penetration is a function of the polar head group and the hydrocarbon chain length.

- **Anionic Surfactants:** e.g. Dioctylsulpho - succinate, Sodium lauryl sulphate, Decodecyl- methyl sulphoxide etc. Nonionic Surfactants: e.g. Pluronic F127, Pluronic F68, etc
- **Bile Salts:** e.g. Sodium mstaurocholate, Sodium deoxycholate, Sodium tauroglycocholate
- **Binary system:** These systems apparently open up the heterogeneous multilaminate pathway as well as the continuous pathways.e.g. Propylene glycol-oleic acid and 1, 4-butane diollinoleic acid

C. Miscellaneous Chemicals

These include urea, a hydrating and keratolytic agent, N, N-dimethylm-toluamide, calcium thioglycolate, anticholin- ergic agents.

Some potential permeation enhancers have recently been described but the available data on their effectiveness sparse. These include eucalyptol, di-o-methyl- ßcyclodextrin and soyabean casein

4. Other Excipients

A. Adhesives: The fastening of all transdermal devices to the skin has so far been done by using a pressure sensitive adhesive which can be positioned on the face of the device and in the back of the device and extending peripherally.

Both adhesive systems should fulfill the following criteria

- Should adhere to the skin aggressively, should be easily removed
- Should not leave an un washable residue on the skin
- Should not irritate or sensitize the skin

The face adhesive system should also fulfill the following criteria

- Physical and chemical compatibility with the drug, excipients and enhancers of the device of which it is a part
- Permeation of drug should not be affected
- The delivery of simple or blended permeation enhancers should not be affected

B. Backing membrane: Backing membranes are flexible and they provide a good bond to the drug reservoir, prevent drug from leaving the dosage form through the top, and accept printing.

It is impermeable substance that protects the product during use on the skin e.g. metallic plastic laminate, plastic backing with absorbent pad and occlusive base plate (aluminium foil), adhesive foam pad (flexible polyurethane) with occlusive base plate (aluminium foil disc) etc.

Desirable features for transdermal patches

- Composition relatively invariant in use
- System size reasonable
- Defined site for application
- Application technique highly reproducible
- Delivery is (typically) zero order
- Delivery is efficient

1.6.6. Factors affecting transdermal drug delivery

Skin condition

The intact skin itself acts as a barrier, but many agents like acids and alkali cross the barrier cells and penetrate through the skin. Many solvents open the complex dense structure of the horny layer: solvents like methanol and chloroform remove the lipid fraction, forming artificial shunts through which drug molecules can pass easily.

Skin age

It is seen that the skin of adults and young ones is more permeable than that of the older ones. but there is no dramatic difference. Children show toxic effects because of the greater surface area per unit body weight. Thus, potent steroids, boric acid and hexachlorophene have produced severe side-effects.

Physicochemical factors

Hydration of skin

Generally, when water saturates the skin, it swells tissues, softens wrinkles on the skin and its permeability increases for the drug molecules that penetrate through the skin.

Temperature and pH of the skin

The penetration rate varies if the temperature varies and the diffusion coefficient decreases as the temperature falls however adequate clothing on the body prevents wide fluctuations in temperature and penetration rates. According to pH, only unionized molecules pass readily across the lipid membrane, and weak acids and bases dissociate to different degrees according to their pH and pKa or pKb values. Thus, the concentration of unionized drug in applied phase will determine the effective membrane gradient, which is directly related to its pH.

Environmental factors

Sunlight

Because of to sunlight, the walls of blood vessels become thinner, leading to bruising, with only minor trauma in the sun-exposed areas. Also, pigmentation, the most noticeable sun-induced pigment change, is a freckle or solar lentigo.

Cold season

The cold season often results in itchy and dry skin. The skin responds by increasing oil production to compensate for the weather's drying effects. A good moisturizer will help ease symptoms of dry skin. Also, drinking lots of water can keep your skin hydrated and looking radiant.

Air pollution

Air pollution can clog pores and increase bacteria on the face and the surface of skin, both of which lead to acne or spots, which affects drug delivery through the skin. Invisible chemical pollutants in the air can interfere with the skin's natural protection system, breaking down the skin's natural oils that normally Dust trap moisture in the skin and keep it supple.

1.6.7. Types of Transdermal Patches [24]

There are four Major Transdermal Systems:

1. **Single-layer Drug-in-Adhesive**

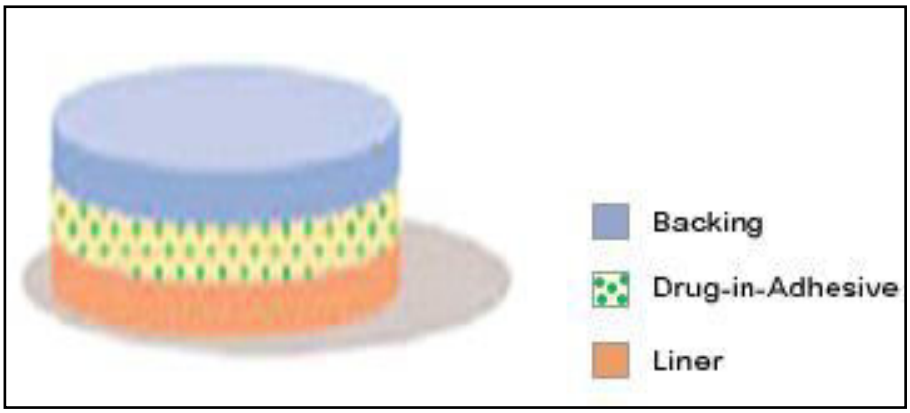


Fig No. 6: Single-layer Drug-in-Adhesive system

The Single-layer Drug-in-Adhesive system is characterized by the inclusion of the drug directly within the skin-contacting adhesive. In this transdermal system design, the adhesive not only serves to affix the system to the skin, but also serves as the formulation foundation, containing the drug and all the excipients under a single backing

film. The rate of diffusion of drug from this type of system is dependent on the diffusion across the skin . The intrinsic rate of drug diffusion from this type of drug delivery system is defined by

$$dQ/Dt = Cr/(1/Pm+1/Pa)$$

Where Cr is the drug concentration in the reservoir compartment and Pa and Pm are the permeability coefficients of the adhesive layer and the rate controlling membrane,

Pm is the sum of permeability coefficients simultaneous penetrations across the pores and the polymeric material. Pm and Pa, respectively, are defined as follows.

$$Pm = K_{m/r}Dm/hm$$

$$Pa = K_{a/m}.Da/ha$$

Where Km/r and Ka/m are the partition coefficients for the interfacial partitioning of drug from the reservoir to the membrane and from the membrane to adhesive respectively, Dm and Da are the diffusion coefficients in the rate controlling membrane and adhesive layer, respectively, and hm and ha are the thicknesses of the rate controlling membrane and adhesive layer, respectively.

2. Multi-layer Drug-in-Adhesive

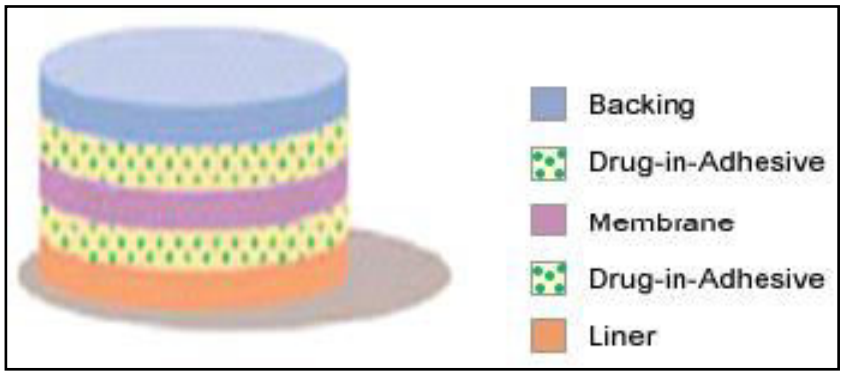


Fig No. 7: Multi-layer Drug-in-Adhesive system

The Multi-layer Drug-in-Adhesive is similar to the Single-layer Drug-in-Adhesive in that the drug is incorporated directly into the adhesive. However, the multi-layer encompasses either the addition of a membrane between two distinct drug-in-adhesive layers or the addition of multiple drug-in-adhesive layers under a single backing film.

The rate of drug diffusion in this system is defined by:

$$Dq/dt = K_{a/r}.D_a /ha (cr)$$

Where Ka/r is the partition coefficient for the interfacial partitioning of the drug from the reservoir layer to adhesive layer.

3. Drug Reservoir-in-Adhesive

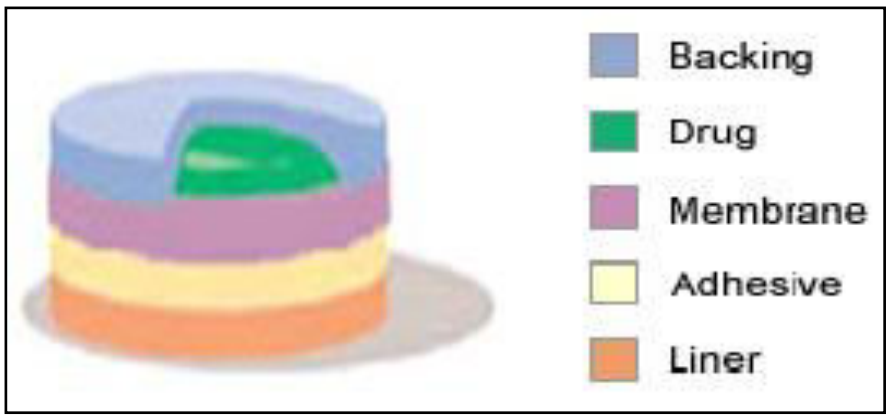


Fig No.8: Drug Reservoir-in-Adhesive system

The Reservoir transdermal system design is characterized by the inclusion of a liquid compartment containing a drug solution or suspension separated from the diffusion liner by a semi-permeable membrane and adhesive. The adhesive component of the product responsible for skin adhesion can either be incorporated as a continuous layer between the membrane and the diffusion liner or in a concentric configuration around the membrane.

The rate of drug diffusion from this drug reservoir gradient controlled system is given by:

$$Dq/dt = K_{a/r}.Da / ha(t).A(ha)$$

In the above equation, the thickness of the adhesive layer for drug molecule to diffuse through increases with time ha (t). To compensate for this time dependent increase in the diffusional path due to the depletion of drug dose by diffusion, the drug loading level is also increased with the thickness of diffusional path A (ha).

4. Drug Matrix-in-Adhesives

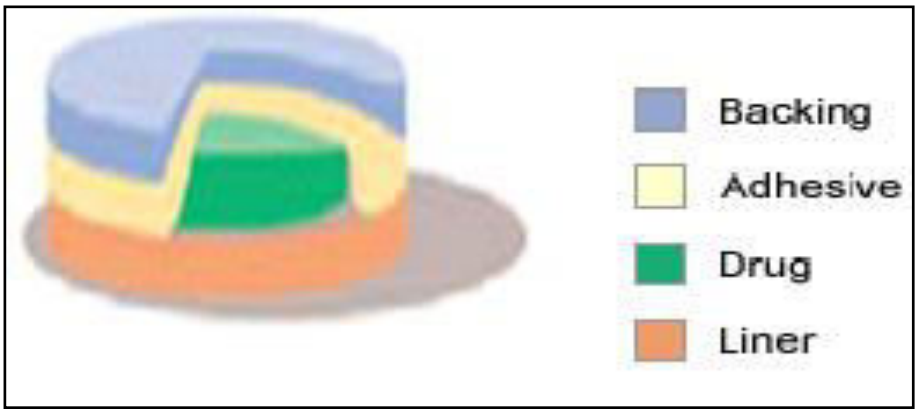


Fig No.9: Drug Matrix-in-Adhesive system

The Matrix system design is characterized by the inclusion of a semisolid matrix containing a drug solution or suspension which is in direct contact with the diffusion liner. The component responsible for skin adhesion is incorporated in an overlay and forms a concentric configuration around the semisolid matrix.

The rate of drug diffusion from this type of system is defined as:

$$dQ / dt = AC_pD_P^{1/2} / 2t$$

Where A is the initial drug loading dose dispersed in the polymer matrix and Cp and Dp are the solubility and diffusivity of the drug in the polymer respectively. Since, only the drug species dissolved in the polymer can diffusion, Cp is essentially equal to CR, where CR is the drug concentration in the reservoir compartment.

1.6.8. Limitations of Transdermal Delivery System

- Higher molecular weight candidates (>500Daltons) fail to penetrate the *Stratum corneum*
- Drugs with very low or high partition coefficient fail to reach systemic circulation
- High melting drugs are not suitable due to their low solubility both in water and fat

- Possibility of local irritation at the site of patch application
- A lag time associated with the delivery of the drug across the skin, resulting in a delay in onset of action
- Variation of absorption rate based on site of application
- Presence of skin diseases
- Variation in adhesive effectiveness in different individuals

1.7. EVALUATION METHODS[25]

The evaluation methods for transdermal dosage form can be classified into following types:

- Physicochemical evaluation
- *In vitro* evaluation
- *In vivo* evaluation

1.7.1. Physicochemical evaluation

1. Interaction studies

The drug and the excipients must be compatible with one another to produce a product that is stable. The interaction between drug and excipients affect the bioavailability and stability of the drug. If the excipients are new and have not been used in formulations containing the active substance, the compatibility studies play an important role in formulation development. Interaction studies are taken out by Thermal analysis, Fourier transform infrared spectroscopy (FTIR), ultra violet (UV) and chromatographic techniques by comparing their physicochemical properties like assay, melting point, wave numbers, and absorption maxima.

2. Thickness of the patch

The thickness of the drug prepared patch is measured by using a digital micrometer at different point of patch and this determines the average thickness and standard deviation for the same to ensure the thickness of the prepared patch.

3. Weight uniformity

The prepared patches are to be dried at 60°C for 4 h before testing. A specified area of patch is to be cut in different parts of the patch and weighed in digital balance. The average weight and standard deviation values are to be calculated from the individual weights.

4. Folding endurance

A specific area of strip is cut and repeatedly folded at the same place till it broke. The number of times the film could be folded without breaking gave the value of folding endurance.

5. Percentage moisture content

The prepared patches are to be weighed individually and to be kept in a desiccator containing fused calcium chloride at room temperature. After 24 h, the films are to be reweighed and the percentage moisture content determined by below formula,

Percentage moisture content (%) = [Initial weight - Final weight / Final weight] ×100

6. Percentage moisture uptake

The prepared patches are to be weighed individually and to be kept in a desiccators containing saturated solution of potassium chloride in order to maintain 84% Rhesus factor (RH). After 24 h, the films are to be reweighed and the percentage moisture uptake determined by the formula.

Percentage moisture uptake (%) = (Final weight - Initial weight / initial weight) × 100

7. Water vapour permeability (wvp) evaluation

Water vapour permeability can be determined by a natural air circulation oven. The WVP can be determined by the following formula.

WVP = W/A Where, WVP is expressed in g/m2 per 24 h, W is the amount of vapour permeated through the patch expressed in g/24 h, A is the surface area of the exposure samples expressed in m.

8.Drug content

A specified area of patch is to be dissolved in a suitable solvent in specific volume. Then, the solution is to be filtered through a filter medium and the drug content analyzed with the suitable method (UV or HPLC technique).Then, the average of three different samples is taken.

9. Content uniformity test

Ten (10) patches were selected and content determined for individual patches. If 9 out of 10 patches have content between 85 to 115% of the specified value and one has content not less than 75 to 125% of the specified value, then transdermal patches pass the test of content uniformity. But if 3 patches have content in the range of 75 to 125%, then additional 20 patches are tested for drug content. If these 20 patches have range from 85 to 115%, then the transdermal patches pass the test.

10. Flatness test

Three longitudinal strips were cut from each film at different portion like one from the center, other one from the left side, and another one from the right side. The length of each strip was measured, and the variation in length because of non-uniformity in flatness was measured by determining percentage constriction, with 0% constriction equivalent to 100% .

$$\text{Flatness Constriction (\%)} = I1 - I2 \times 100\ I1$$

Where, I1 = initial length of each strip. I2 = final length of each strip.

11. Percentage elongation break test

The percentage elongation break was determined by noting the length just before the break point and determined from the formula.

$$\text{Elongation percentages} = L1 - L2 / L2 \times 100$$

Where L1 = final length of each strip L2 = initial length of each strip

12. Peel Adhesion Properties

Peel adhesion is the force required to remove all adhesive coating from test substrate, its important in transdermal devices because the adhesive should provide adequate contact of device with the skin of the adhesive polymer, the type and amount of adhesive and polymer composition. It's tested by measuring the force required to pull a single coated tape, applied to substrate, at a 180º angle, No residue on the substrate indicates adhesive failure which is desirable for transdermal devices, remnants on substrate indicates cohesive failure. Signifying a deficit of cohesive strength in the coating as shown in Fig No:10

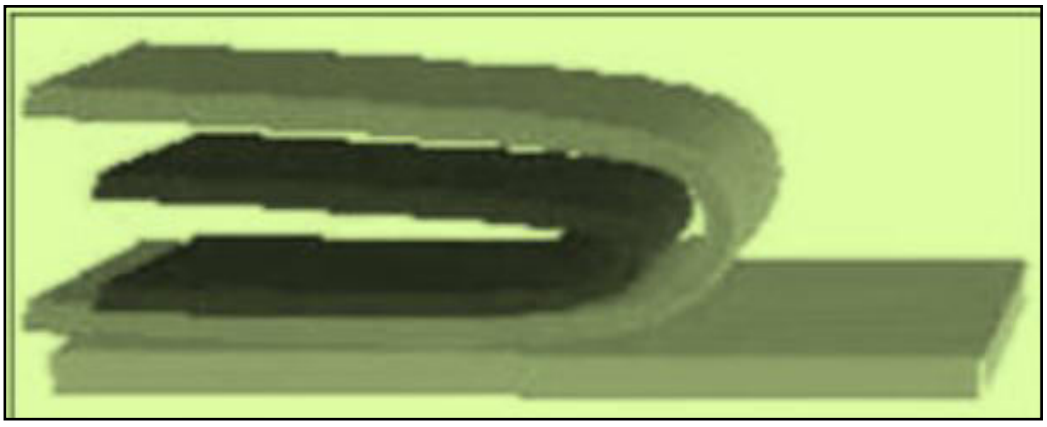


Fig No.10: Peel Adhesion Properties

13. Tack Properties

Tack is the ability of a polymer to adhere to substrate with little contact pressure. It is important in transdermal devices which are applied with finger pressure. Tack is dependent on the molecular weight and composition of polymer as well as use tackifying resins in the polymer.

14.Thumb Tack Test

This is subjective test in which evaluation is done by pressing the thumb briefly into the adhesive experience is required for using test.

15.Rolling Ball Tack Test

This test involves measurement of distance travelled by a stainless steel along the upward face of adhesive. The diameter of ball is 7/16o inches and it diffusiond on inclined track having angle 22.5 or more the distance travelled, less the tacky polymer. Distance travelled by ball is measured in inches which determine the tackiness of polymer. It determines the softness of adhesive polymer. As shown in Fig No.11

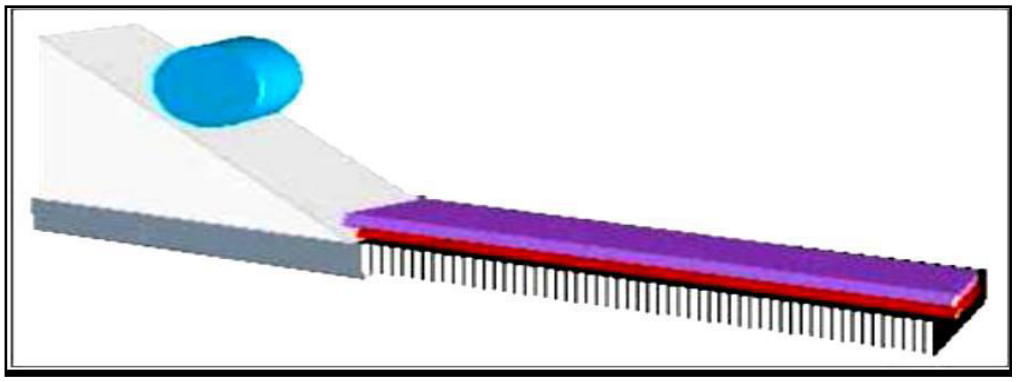


Fig No.11:Rolling Ball Tack Test

16.Quick-Stick (Or Peel-Tack) Test

The peel force required to break the bond between an adhesive and substrate is measured by pulling the tape away from the substrate at 90° at a speed of 12 inch/min. The force is recorded as the tack value and expressed in ounces (or grams) per inch width with higher values indicating increasing tack. As shown in Fig No.12

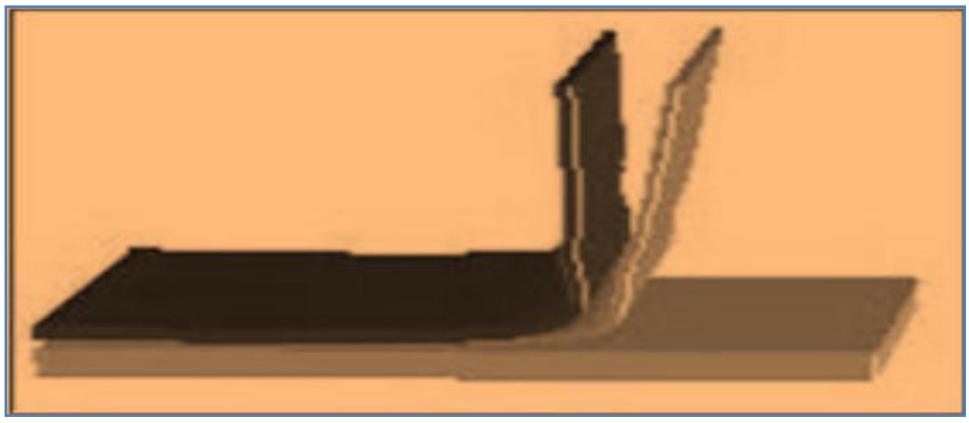


Fig No.12: Quick-Stick (Or Peel-Tack) Test

17.Probe Tack Test

In this, the tip of probe with defined surface roughness brought in to contact with adhesive and when the bond is formed between the adhesive a probe, removal of probe at a fixed rateaway from the adhesive which break the bond. The force required to break the bond Is recorded as tack and it is expressed in grams as shown in Fig No. 13

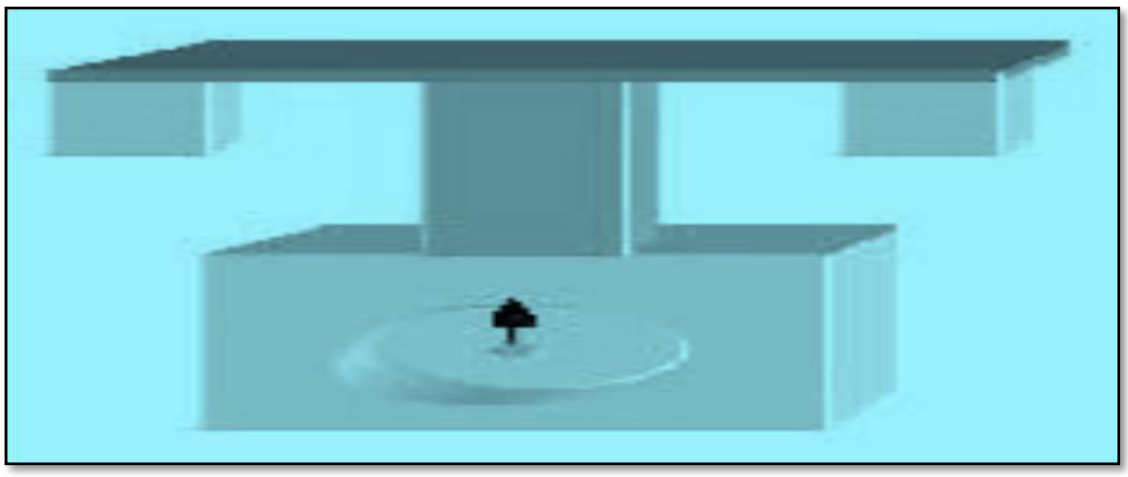


Fig No. 13. Probe tack test

18. Shear strength Properties

The cohesive strength of an adhesive polymer is determined by this test. The value of strength can be affected by the degree of cross linking, the molecular weight, the composition of polymer and the amount of tackifiers added. An adhesive coated patch is stacked on plate made of stainless steel and specified weight hung from the patch parallel to this plat. The time taken to pull off the patch from the plate determines the cohesive strength. More the time taken, greater is the shear strength as shown in Fig no14.

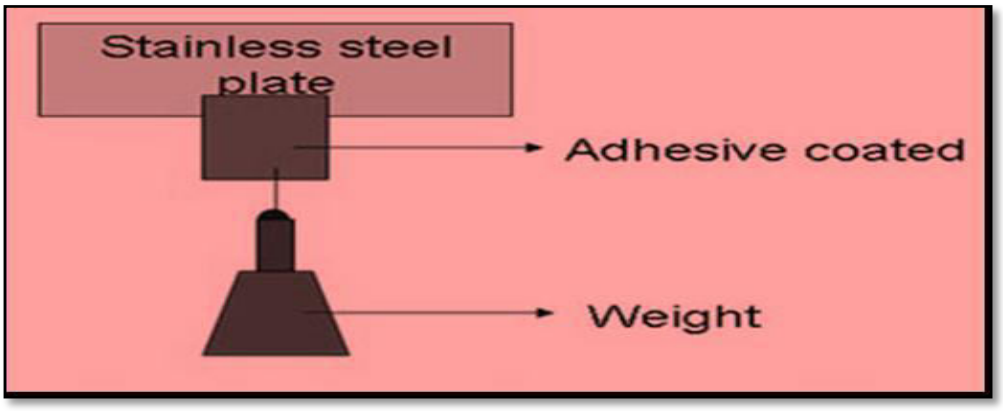


Fig No.14. Shear strength test

19. Stability studies

Stability studies were conducted according to the International Conference on Harmonization (ICH) guidelines by storing the TDDS samples at 40 ± 0.5°C and 75 ± 5% RH for 6 months. The samples were withdrawn at 0, 30, 60, 90 and 180 days and analyzed suitably for the drug content.

1.7.2. *In vitro* evaluation of TDDS [26]

In vitro drug diffusion studies

The paddle over disc method (USP apparatus) can be employed for assessment of the diffusion of the drug from the prepared patches. Dry films of known thickness were cut into definite shape, weighed, and fixed over a glass plate with an adhesive. The glass plate was then placed in a 500 ml of the dissolution medium or phosphate buffer (pH 7.4), and the apparatus was equilibrated to 32 ± 0.5°C. The paddle was then set at a distance of 2.5 cm from the glass plate and operated at a speed of 50 rpm. Samples (5 ml aliquots) can be withdrawn at appropriate time intervals up to 24 h and analyzed by UV spectrophotometer or HPLC. The experiment was performed in triplicate and the mean value calculated.

Flux was determined directly as the slope of the curve between the steady-state values of the amount of drug permeated (mg cm2) versus time in hours, and permeability coefficients were deduced by dividing the flux by the initial drug load (mg cm2).

2. a. Literature review on *Azadirachta indica* A.juss (Neem)

Mohammad A. Alzohairy *et al.,* (2016) studied the therapeutic role of *Azadirachta indica* (Neem) and their active constituents in diseases prevention and treatment. Neem (*Azadirachta indica*) is a member of the Meliaceae family and its role as health-promoting effect is attributed because it is rich source of antioxidant. It has been widely used in Chinese, Ayurvedic, and Unani medicines worldwide especially in Indian Subcontinent in the treatment and prevention of various diseases. its constituents play role in the scavenging of free radical generation and prevention of disease pathogenesis

Chhibber.S *et al.,* (2014) reviewed the medicinal and therapeutic use of *Azadirachta indica,* a fast growing, evergreen tree found commonly in India, Africa and America. Neem is one of the most useful traditional medicinal plant in India. It is a highly esteemed tree with several beneficial properties and applications, especially known for its incredible therapeutic and ethnomedicinal values for mankind. It has been used in different medicinal systems: Ayurveda, unani, homeopathic medicine, therefore considered as cynosure of modern medicine

Soniya Adyanthaya *et al.,* (2014) evaluated the antimicrobial potential of the extracts of the twigs of *Azadirachta indica* (Neem). An *in vitro* study of various medicinal plants have been used since ancient times in daily life to treat diseases all over the world. Almost every part of the *Azadirachta indica* A. Juss (syn. *Melia azadirachta*) tree commonly known as neem has been used for years to treat a number of human diseases. As neem is known to possess antimicrobial activity against a number of organism**s**

Jaya vikas kurhekar *et al*., (2013) analysed the aqueous and acetone extracts of neem a for inherent components, anti-microbial activities against common pathogens and compared with standard antibiotics

Imam Hashmat *et al.,* (2012) reviewed that *Azardirachta indica A juss* is a nature's drug store. This review gives a bird's eye view mainly on the biological activities of the neem and some of their compounds isolated, pharmacological actions of the neem extracts, clinical studies and medicinal applications of neem along with their safety evaluation

Sugumari Elavarasu *et al.,* (2012) evaluated anti-plaque microbial activity of *Azadirachta indica* (neem oil) *in vitro*: The use of *Azadirachta indica* (Indian neem) is considered as the plants have a wide spectrum of bioactivity. They are used as antibacterial, antifungal, and anticancerous agents. The purpose of the study was to utilize this Indian herb in the reduction of microorganisms

Kausik Biswas *et al.,* (2011) reviewed that biological activities and medicinal properties of neem (*Azadirachta indica* A. Juss) is perhaps the most useful traditional medicinal plant in India. Each part of the neem tree has some medicinal property and is thus commercially exploitable. During the last five decades, apart from the chemistry of the neem compounds, considerable progress has been achieved regarding the biological activity and medicinal applications of neem. It is now considered as a valuable source of unique natural products for the development of medicines against various diseases

Alok Maithani *et al.,* (2011) reviewed *Azadirachta indica* (Neem) leaf is one of the most revenue releasing plant grown in India because of several phytoconstituents present in it and also due to a number of pharmacological activities associated with it. The present review highlights a literature on taxonomical, botanical, phytoconstituents, and pharmacological discussion on *Azadirachta indica* leaves

A. Biu *et al*., (2009) done phytochemical screening of *Azadirachta indica* (Neem) (Meliaceae). Air dried aqueous-extract has been subjected to screen for some active chemical constituents. Saponins had high scores in the extract, tannins and glycosides indicated moderate scores, while alkaloids, terpenes, flavonoids, reducing sugars, pentoses and whole carbohydrates showed low scores. Anthraquinones, ketones and mono saccharides were not detected from the extract

Mary Ndung'u *et al*., (2004) studied that Neem (*Azadirachta indica* A. Juss) is known to be an important source of triterpenoids; and multipurpose tree species native to the dry forest zones of the Asian subcontinent including India, Sri Lanka, Pakistan, Bangladesh, Malaysia, Myanmar and Thailand. The tree is found both cultivated and growing in its natural habitat in this zones and it has been transferred to many of the warmer parts of the world.

2. LITERATURE REVIEW

2. b. Literature Review on TDDS

- **L. Karpagavalli *et al.,* (2017)** formulated and evaluated Transdermal Patches of Curcumin. The work showed that drug of ayurvedic origin can be utilized in a better form. The films formed using HPMC were thin, flexible and smooth and these patch with permeation enhancer showed maximum enhanced efficacy for incorporation into modern dosage forms. The sustained release of curcumin from transdermal patches can minimize the frequent administration of conventional curcumin dosage forms

- **Suneetha Cherukuri *et al.,* (2017)** formulated and evaluated of transdermal drug delivery of Topiramate. On the basis of results obtained from the physical evaluation and *ex vivo* studies, the patches containing the polymers, Eudragit L 100 and polyvinylpyrrolidone, with oleic acid as the penetration enhancer were considered as the best formulations for the transdermal delivery of (TPM)

- **Deepak Kumar Patel *et al.,* (2016)** formulated transdermal patch of curcumin with the main objective to minimize the side effects and maximize the therapeutic efficacy. The formulations were evaluated for weight variation, moisture uptake, thickness, moisture content and folding endurance

- **Shashi Kumar Yadav *et al.,* (2013)** formulated and evaluated of transdermal patch of Ayurvedic Antirheumatic Drug using Different polymers . 4 - day skin permeation study shows 81.44% release of drug from the formulation 7 containing polymers HPMC, Ethyl cellulose and PEG-6000 (10:10:1). Hence, this formula was considered to be the optimized formulation with good physicochemical properties, skin compatibility, and sustained drug release

- **Sunil R. Rathva *et al.*, (2012)** carried out a review on Herbal transdermal patches. It has been found that drugs from herbal origin can be utilized with enhanced efficacy by incorporating in transdermal patches. Herbal transdermal patches aids to quit smoking, relieve stress, increase sexuality, insect repellant patches, detoxification, male energizer, postpone menopause are available in market

- **Kansagra Hemanh *et al.*, (2012)** formulated and evaluated transdermal patch of Sertaconazole nitrate. The permeation studies illustrated that the ratio of polyvinyl pyrrolidone and ethyl cellulose 1:5 showed good controlled release. Higuchi and Korsmeyer-Peppas models were used for optimizing the formulation

- **Santosh S Bhujbal *et al.*, (2011)** developed a novel herbal formulation in the management of diabetes .The weight of transdermal patches of *M. charantia* (2 cm^2; 10 mg/ patch) and was found to be 0.03 gm. Thickness of patches of *M. charantia* (2 cm^2; 10 mg/patch) was found to be satisfactory. The percentage release of active constituents from transdermal patches of *M.charantia* (2 cm^2; 10 mg/ patch) was found to be 47.59% in 10% hydroalcoholic phosphate buffer pH 7.4 at the end of 6 h

- **Sarswathi R *et al.*, (2010)** formulated and evaluated transdermal patches of Curcumin. The *in - vitro* permeation studies of patches was carried out using 0.5% Sodium Lauryl Sulphate solution in the receptor compartment with their different patches, F1, F2 and F3 using HPMC and EC bring the satisfactory release of curcumin. The cumulative percentage drug release of patch F1-82.20%, F2-74.06% and F3-68.27%. The release kinetics was evaluated making by use of Zero order, first order, Higuchi's diffusion and Korsemeyer-Peppas equation. The drug release through transdermal patches of curcumin followed Zero order kinetics

3. AIM AND OBJECTIVE

3.1. Aim

To prepare and evaluate Transdermal Patch of Aqueous extract of *Azadirachta indica* A. juss using different polymers

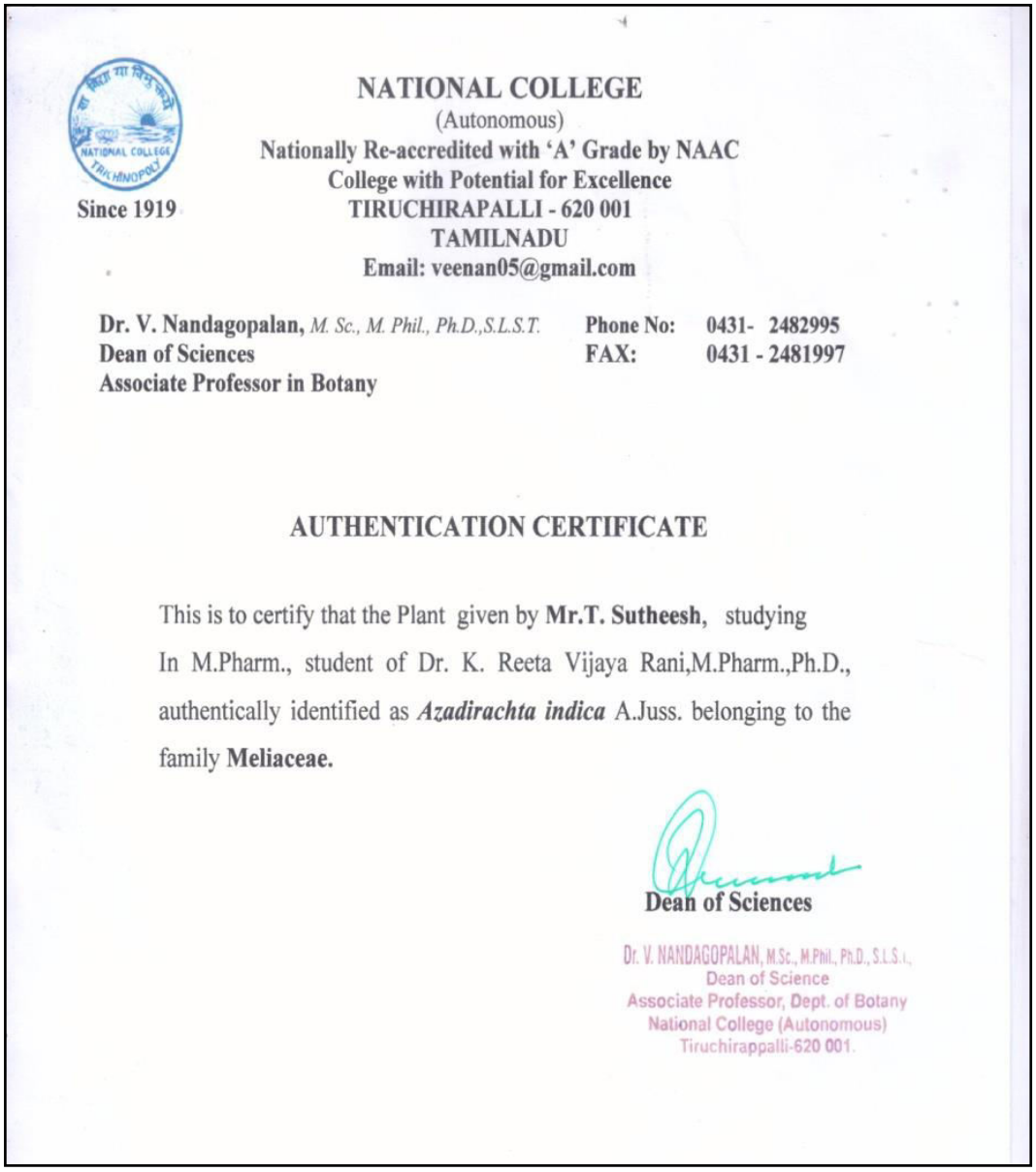
3.2. Objective

- To convert the herbal extract into a novel dosage form
- To provide a direct entry of aqueous extract into the systemic circulation to give sustained action
- To develop a novel topical formulation of aqueous extract of neem leaves for the effective treatment of Skin infections

4. PLAN OF THE WORK

- Literature Survey
- Selection of Herb
- Procurement of dried powder of leaves of *Azadirachta indica* A. juss.
- Phytochemical evaluations
- Selection of Polymers
- Trial batches for Transdermal Patches (extract : polymer in different ratio)
- Evaluations
    - **Physico chemical evaluation**
        - ✓ Percent moisture absorption
        - ✓ Percent moisture loss
        - ✓ Thickness
        - ✓ Weight variation
        - ✓ Folding endurance
        - ✓ Surface pH
        - ✓ Drug content
    - *In- vitro* Evaluation
    - Anti-Microbial screening studies
    - Release kinetics
    - *Ex-vivo* studies
    - Stability studies
- Results and discussions
- Summary and Conclusion

PLANT AUTHENTICATION CERTIFICATE



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Dean of Sciences
Associate Professor in Botany

Phone No: 0431- 2482995
FAX: 0431 - 2481997

AUTHENTICATION CERTIFICATE

This is to certify that the Plant given by **Mr.T. Sutheesh,** studying In M.Pharm., student of Dr. K. Reeta Vijaya Rani,M.Pharm.,Ph.D., authentically identified as ***Azadirachta indica*** A.Juss. belonging to the family **Meliaceae.**

Dean of Sciences

Dr. V. NANDAGOPALAN, M.Sc., M.Phil., Ph.D., S.L.S.T.,
Dean of Science
Associate Professor, Dept. of Botany
National College (Autonomous)
Tiruchirappalli-620 001.

Fig No.15: Authentication of plant

5.1. PLANT PROFILE

Fig No.16: Leaves of *Azadirachta indica* A. Juss.

5.1.1. Scientific Classification [28]

Kingdom	**:**	Plantae
Order	**:**	Sapindales
Family	**:**	Meliaceae
Genus	**:**	*Azadirachta*
Species	**:**	*indica*
Common name	**:**	Neem
English	**:**	Neem
Tamil	**:**	Veppai, vembu,sengumaru
Hindi	**:**	Neem

5.1.2. Description

Colour	**:**	Green
Taste	**:**	Bitter
Odour	**:**	Pungent

5.1.3. Synonyms

- *Azadirachta indica var. minor*

- *Azadirachta indica var. siamensis*
- *Azadirachta indica subsp. Vartakii*
- *Melia azadirachta* L.
- *Melia indica* (A. Juss.)

5.1.4. Habitat [29]

Most well-known for its insecticidal properties, the neem tree (*Azadirachta indica*) grows as a statuesque woodland, shade and crop tree in many tropical and subtropical countries. As well as its many practical uses, it's also ornamental, bearing 10-inch-long, fragrant, white, spring flowers. Neem tree's habitats include its natural growing range and the many areas where humans have planted it, taking advantage of its tolerance for tough conditions. It is hardy in U.S. Department of Agriculture plant hardiness zones 10 to 12.

5.1.5. Vernacular names of *Azadirachta indica* A. Juss.

- ✓ Tamil - Veppai (□□□□□□), Sengumaru
- ✓ English - Margosa, Neem Tree
- ✓ Hindi – Neem
- ✓ Kannada – Bevu
- ✓ Malayalam – Aryaveppu
- ✓ Sanskrit - Arishta, Pakvakrita, Nimbaka
- ✓ Telugu – Vepa

5.1.6. Uses [30,31]

Azadirachta indica, commonly known as neem, has attracted worldwide prominence in recent years, owing to its wide range of medicinal properties. Neem has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine. Neem elaborates a vast array of biologically active compounds that are chemically diverse and structurally complex. More than 140 compounds have been isolated from different parts of neem. All parts of the neem tree - leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. The medicinal utilities have been described especially for neem leaf. Neem leaf and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial,

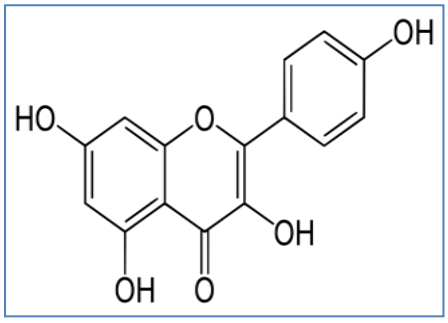
antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties.

5.1.7.Pharmacological activity[32]

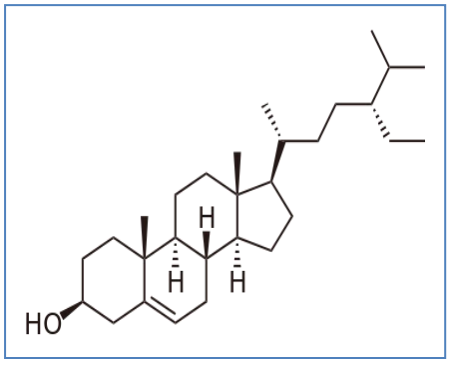
- ✓ Analgesic agent
- ✓ Antipyretic agent
- ✓ Antimicrobial activity
- ✓ Antibacterial activity
- ✓ Antifungal activity
- ✓ Antiviral activity
- ✓ Contraceptic
- ✓ Hepatoprotective
- ✓ Anti-hyper glycemic agent

5.1.8. Chemical constituents [33,34]

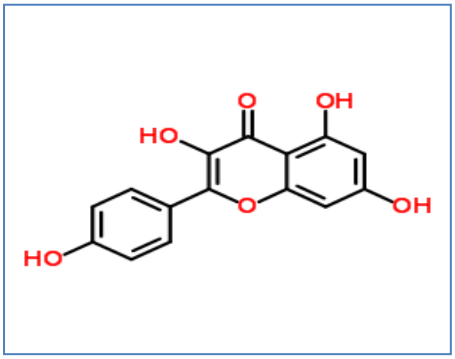
Azadiractin



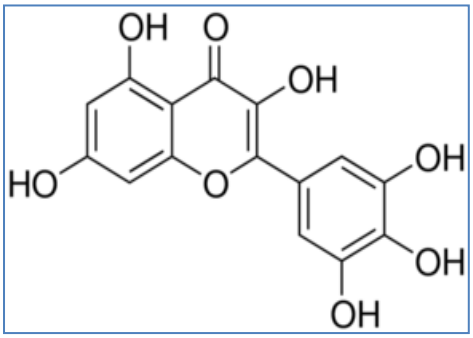
β- sitosterol



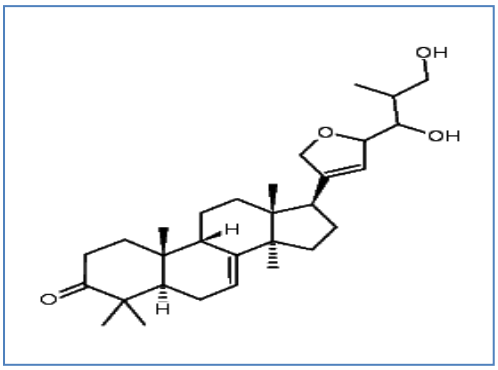
Kaempferol



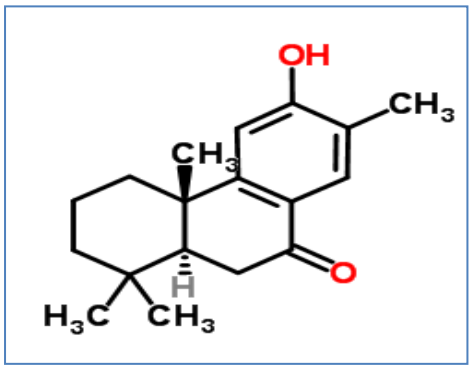
Myrecetin



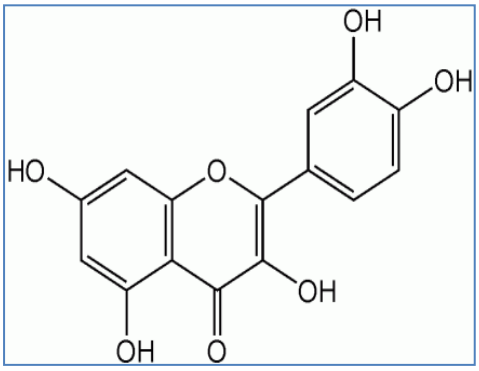
Nimbidinine



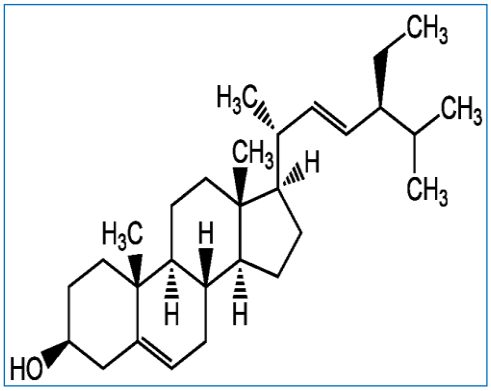
Nimbiol



Quercetin



Stigmasterol



5.2. EXCIPIENT PROFILE [35,36]

5.2.1. Pectin

Pectin is structural hetero-polysaccharides contained in the primary cell walls of terrestrial plants. Pectin is a naturally occurring biopolymer that is finding increasing applications in the pharmaceutical and biotechnology industry. It has been used successfully for many years in the food and beverage industry as a thickening agent, a gelling agent and a colloidal stabilizer. Pectin also has several unique properties that have enabled it be used as a matrix for the entrapment and or delivery of a variety of drugs, proteins and cells. This review will first describe the source and production, chemical structure and general properties of pectin. The methods of gel formation and properties of the gels will then be discussed.

Physical properties : White to light brown powder

Chemical Structure :

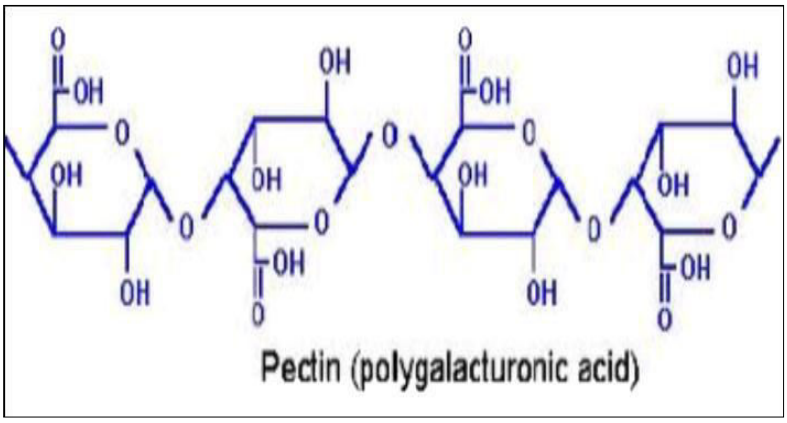


Fig No.17: Pectin

Molecular weight : 60,000-130,000 g/ mol

Uses : Gelling agent, thickening agent, stabilizer in Food

5.2.2. Sources of Pectin

- Apples – 1 to 1.5%
- Apricot – 1%
- Cherries – 0.4%
- Oranges – 0.5 to 3.5%
- Carrots approx – 1.4%
- Citrus peels – 53%

5.2.3. General properties of Pectin

Pectin is a natural polymer. Pectin is soluble in pure water. Monovalent cation (alkali metal) salts of pectinic and pectic acids are usually soluble in water, di- and trivalent cations salts are weakly soluble or insoluble. Dry powdered pectin, when added to water, has tendency to hydrate very rapidly, forming clumps. These crumps consist of semi dry packets of pectin contained in an envelope of highly hydrated outer coating. Further solubilisation of such cramps is very slow. Dilute pectin solutions are Newtonian but a moderate concentration, they exhibit the non-Newtonian, pseudo plastic behavior characteristics. As with solubility, the viscosity of a pectin solution is related to the molecular weight, concentration of the preparation, and the pH and presence of counter ions in the solution. Viscosity, solubility and gelation are generally related. For example, factors that increase gel strength will increase the tendency to gel, decrease solubility, and increase viscosity, and vice versa.

5.2.4. Pharmaceutical uses of pectin

- ✓ Pectin has applications in the pharmaceutical industry. Pectin favorably influences cholesterol levels in blood. Consumption of at least 6g/day of pectin is necessary to have a significant effect in cholesterol reduction
- ✓ Pectin acts as a natural prophylactic substance against poisoning with toxications. It has been shown to effective in removing lead and mercury from the gastrointestinal tract and respiratory organs
- ✓ Pectin injected intravenously, shortens the coagulation time of drawn blood, thus being useful in controlling hemorrhage or local bleeding

- ✓ Pectin and combinations of pectin with other colloids have been used extensively to treat diarrheal diseases, especially in infants and children
- ✓ Pectin reduces rate of digestion by immobilizing food components in the intestine. This results in less absorption of food
- ✓ Pectin hydrogels have been used in tablet formulations as a binding agent matrix tablet formulations. HM-pectin's for their potential value in controlled diffusion matrix formulations. The application of a binary polymer system, (i.e) HM-pectin and hydroxypropyl methylcellulose
- ✓ Pectin beads prepared by the ionotropic gelation method were used as a sustained diffusion drug delivery system
- ✓ Pectin has a promising pharmaceutical uses and is presently considered as a carrier material in colon-specific drug delivery systems (for systemic action or a topical treatment of diseases such as ulcerative colitis, crohn's disease, colon carcinomas)
- ✓ Pectin is an interesting candidate for pharmaceutical use, e.g. as a carrier of variety of drugs for controlled diffusion applications. Many techniques have been used to manufacture the pectin-based delivery systems, especially ionotropic gelation and gel coating

5.3. Sodium alginate

Nonproprietary Names

BP **:** Sodium alginate

PhEur **:** Natrialginas

USPNF **:** Sodium alginate

Synonyms

Algin; alginic acid, sodium salt; E401; *Kelcosol; Keltone; Protanal;* sodium polymannuronate.

5.3.1. Structure of Sodium alginate

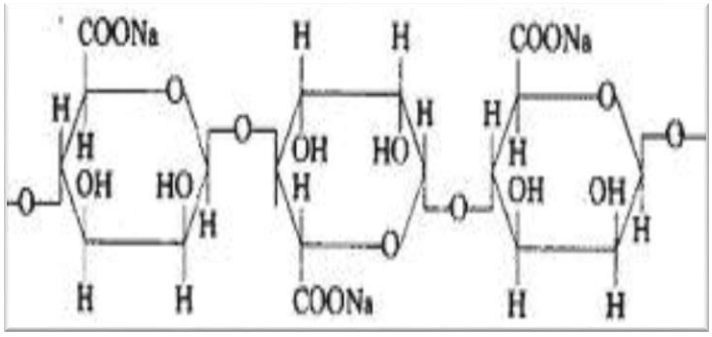


Fig No.18: Sodium alginate

Chemical Name and CAS Registry Number

Sodium alginate [9005-38-3]. Sodium salt of alginic acid, which is a mixture of polyuronic acids composed of residues of D-mannuronic acid and L-guluronic acid. The block structure and molecular weight of sodium alginate samples have been investigated

5.3.2. Functional Category

Stabilizing agent; suspending agent; tablet and capsule disintegrant; tablet binder; viscosity increasing agent.

5.3.3. Applications in Pharmaceutical Formulation or Technology

Sodium alginate is used in a variety of oral and topical pharmaceutical formulations. In tablet formulations, sodium alginate may be used as both a binder and disintegrant; it has been used as a diluent in capsule formulations. Sodium alginate has also been used in the preparation of sustained-diffusion oral formulations since it can delay the dissolution of a drug from tablets, capsules, and aqueous suspensions.

In topical formulations, sodium alginate is widely used as a thickening and suspending agent in a variety of pastes, creams and gels and as a stabilizing agent for oil-in-water emulsions. Recently, sodium alginate has been used for the aqueous microencapsulation of

drugs, in contrast with the more conventional microencapsulation techniques which use organic solvent systems. It has also been used in the formation of nanoparticles.

The adhesiveness of hydrogels prepared from sodium alginate has been investigated and drug diffusions from oral mucosal adhesive tablets, and buccal gels, based on sodium alginate have been reported. Other novel delivery systems containing sodium alginate include ophthalmic solutions that form a gel *in-situ* when administered to the eye; and *in- situ* forming gel containing paracetamol fororal administration; and a freeze-dried device intended for the delivery of bone-growth factors Hydrogel systems containing alginates have also been investigated for delivery of proteins and peptides.

Therapeutically, sodium alginate has been used in combination with an H2-receptor antagonist in the management of gastro esophageal reflux, and as a haemostatic agent in surgical dressings. Alginate dressings, used to treat exuding wounds, often contain significant amounts of sodium alginate as this improves the gelling properties. Sponges composed of sodium alginate and chitosan produce a sustained drug diffusion and may be useful as wound dressings or as tissue engineering matrices. Sodium alginate is also used in cosmetics and food products.

5.3.4. Description

Sodium alginate occurs as an odourless and tasteless, white to pale yellowish-brown coloured powder.

5.3.5. Typical Properties

Acidity/alkalinity: pH 7.2 for a 1% w/v aqueous solution.

Solubility

Practically insoluble in ethanol (95%), ether, chloroform, and ethanol/water mixtures in which the ethanol content is greater than 30%. Also, practically insoluble in other organic solvents and aqueous acidic solutions in which the pH is less than 7. Slowly soluble in water, forming a viscous colloidal solution.

Viscosity (dynamic)

Various grades of sodium alginate are commercially available that yield aqueous solutions of varying viscosity. Typically, a 1% w/v aqueous solution, at 20^0C, will have a viscosity of 20-400 m Pas (20-400cP). Viscosity may vary depending upon concentration, pH, temperature, or the presence of metal ions. Above pH 10, viscosity decreases.

5.3.6. Stability and Storage Conditions

Sodium alginate is a hygroscopic material, although it is stable if stored at low relative humidities and a cool temperature.

Aqueous solutions of sodium alginate are most stable at pH 4-10. Below pH 3, alginic acid is precipitated. A 1% w/v aqueous solution of sodium alginate exposed to differing temperatures had a viscosity 60-80% of its original value after storage for 2 years. Solutions should not be stored in metal containers.

Sodium alginate solutions are susceptible on storage to microbial spoilage, which may affect solution viscosity. Solutions are ideally sterilized using ethylene oxide, although filtration using a 0.45µm filter also has only a slight adverse effect on solution viscosity. Heating sodium alginate solutions to temperatures above 70^0C causes depolymerization with a subsequent loss of viscosity. Autoclaving of solutions can cause a decrease in viscosity, which may vary depending upon the nature of any other substances present. Gamma irradiation should not be used to sterilize sodium alginate solutions since this process severely reduces solution viscosity.

Preparations for external use may be preserved by the addition of 0.1% chlorcresol, 0.1% chloroxylenol or parabens. If the medium is acidic, benzoic acid may also be used. The bulk material should be stored in an airtight container in a cool, dry place.

Incompatibilities

Sodium alginate is incompatible with acridine derivatives, crystal violet, phenyl mercuric acetate and nitrate, calcium salts, heavy metals, and ethanol in concentrations greater than 5%. Low concentrations of electrolytes cause an increase in viscosity but high electrolyte concentrations cause salting-out of sodium alginate

Safety

Sodium alginate is widely used in cosmetics, food products, and pharmaceutical formulations, such as tablets and topical products, including wound dressings. It is generally regarded as a nontoxic and non irritant material, although excessive oral consumption may be harmful. A study in five healthy male volunteers fed a daily intake of 175 mg/kg body-weight of sodium alginate for 7 days, followed by a daily intake of 200 mg/kg body-weight of sodium alginate for a further 16 days, showed no significant adverse effects.

The WHO has not specified an acceptable daily intake for alginic acid and alginate salts as the levels used in food do not represent a hazard to health.

Inhalation of alginate dust may be irritant and has been associated with industrial-related asthma in workers involved in alginate production. However, it appears that the cases of asthma were linked to exposure to seaweed dust rather than pure alginate dust.

Handling Precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled. Sodium alginate may be irritant to the eyes or respiratory system if inhaled as dust. So Eye protection, gloves and a dust respirator are recommended. Sodium alginate should be inhaled in a well-ventilated environment.

5.4. Glycerin

5.4.1. Nonproprietary Names

BP : Glycerol

PhEur : Glycerolum

5.4.2. Synonyms

Croderol; E422; glycerine; Glycon G-100; Kemstrene; Optim; Pricerine; 1,2,3-propanetriol; trihydroxypropane glycerol.

5.4.3. Chemical name

Propane 1, 2, 3-triol.

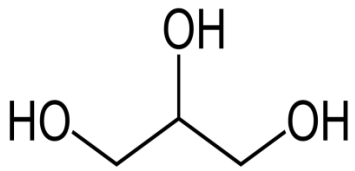
5.4.4. Empirical Formula

$C_3H_8O_3$

5.4.5.Molecular Weight

92.09

5.4.6. Structural Formula



GLYCEROL

5.4.7. Description

Its a clear, colorless, odourless, viscous, hygroscopic liquid, it has a sweet taste, approximately 0.6times as sweet as sucrose.

5.4.8. Functional category

Antimicrobialpreservative,emollient,humectant,plasticizer,solvent.

5.4.9. Typical properties

- **Melting point** : 17.8^0C
- **Solubility** : Soluble in water, methanol

5.4.10. Applications in Pharmaceutical Technology

It is used in a wide variety of pharmaceutical formulations including oral, ophthalmic, topical & parenteral preparations. In topical pharmaceutical formulations and cosmetics, it is used primarily for its humectant & emollient properties. In parenteral formulations it is used mainly as a solvent. In oral solutions, glycerin is used a solvent, sweetening agent, antimicrobial preservative and viscosity increasing agent. It is also used as a plasticizer and in film coatings. It is additionally used in topical formulation such as creams and emulsions. It is used as a plasticizer of gelatin in the production of soft-gelatin capsules and gelatin suppositories. It is employed as a therapeutic agent in a variety of clinical applications, and is also used as a food additive.

5.4.11. Stability and storage conditions

It is hygroscopic. Pure glycerin is not prone to oxidation by the atmosphere under ordinary storage conditions but it decomposes on heating, with the evaluation of toxic acrolein.

Mixture of glycerin with water, ethanol & propylene glycol are chemically stable.It may crystallize if stored at low temperatures; the crystals do not melt until warmed to 20ºC. It should be stored in an airtight container, in a cool, dry place.

5.5. Dimethyl Sulfoxide

5.5.1. Non-proprietary Names

BP : Dimethyl sulfoxide

PhEur : Dimethylis sulfoxidum

USP : Dimethyl sulfoxide

5.5.2. Synonyms

Deltan; dimexide; dimethyl sulphoxide; DMSO; Kemsol; methylsulfoxide; Rimso-50; sulphinyl bismethane

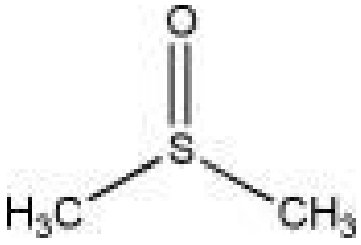
5.5.3. Chemical Name and CAS Registry Number

Sulfinyl bismethane [67-68-5]

5.5.4. Empirical Formula and Molecular Weight

C_2H_6OS M.W: 78.13

5.5.5. Structural Formula



5.5.6. Functional Category

Penetration enhancer; solvent.

5.5.7. Applications in Pharmaceutical Formulation or Technology

Dimethyl sulfoxide is a highly polar substance that is aprotic, therefore lacking acidic and basic properties. It has exceptional solvent properties for both organic and inorganic components, which are derived from its capacity to associate with both ionic species and neutral molecules that are either polar or polarizable. Dimethyl sulfoxide enhances the topical penetration of drugs owing to its ability to displace bound water from the *stratumcorneum* ; this is accompanied by the extraction of lipids and configurational changes of proteins. The molecular interactions between dimethyl sulfoxide and the stratum corneum, as a function of depth and time, have been described. Much of the enhancement capacity is lost if the solvent is diluted. Increases in drug penetration have been reported with dimethyl sulfoxide concentrations as low as 15%, but significant increases in permeability generally require concentrations higher than 60–80%. Furthermore, while low molecular weight substances can penetrate quickly in to the deep layers of the skin, the appreciable transport of molecules with a molecular weight of more than 3000 is difficult. The use of dimethyl sulfoxide to improve transdermal delivery has been reported for cyclosporin,timolol, and a wide range of other drugs. Dimethyl sulfoxide has also been used in the formulation of an injection containing allopurinol. It has also been investigated for use in an experimental parenteral preparation for the treatment of liver tumors. In paint formulations of idoxuridine, dimethyl sulfoxide acts both as a solvent to increase drug solubility and a means of enabling penetration of the antiviral agent to the deeper levels of the epidermis.

5.5.8. Typical Properties

Boiling point

189°C

Dielectric constant

48.9 at 20°C

Solubility

Miscible with water with evolution of heat; also miscible with ethanol(95%), ether and most organic solvents; immiscible with paraffins, hydrocarbons. Practically insoluble in acetone, chloroform, ethanol (95%), and ether.

Vapor pressure

0.37 mm at 20°C

Viscosity (dynamic)

1.1mPa s (1.1 cP) at 27°C

5.5.9. Stability and Storage Conditions

Dimethyl sulfoxide is reasonably stable to heat but upon prolonged reflux it decomposes slightly to methyl mercaptan and bismethyl thio methane. This decomposition is aided by acids, and is retarded by many bases. When heated to decomposition, toxic fumes are emitted.

At temperatures between 40–60°C, it has been reported that dimethyl sulfoxide suffers a partial breakdown, which is indicated by changes in physical properties such as refractive index, density, and viscosity. Dimethyl sulfoxide should be stored in airtight, light-resistant containers.The PhEur 2005 states that glass containers should be used. Contact with plastics should be avoided.

6. MATERIALS AND METHODS

6.1. List of Chemicals

Table No.1. List of Chemicals

S. No.	Chemical Name	Company Name
1.	Pectin	Loba, Mumbai
2.	Sodium alginate	Kemphasol, Bombay
3.	DMSO	Merck Limited, Mumbai
4.	Glycerin	Merk Limited, Mumbai
5.	Potassium dihydrogenortho phosphate	Microfine Chemicals, New Delhi
6.	Sodium hydroxide	Microfine Chemicals, New Delhi

6.2. List of Equipments

Table No.2. List of equipm

S.No.	Name of Equipment	Name of Manufacturer	Purpose
1.	Dessicator	-	Moisture content studies
2.	Digital Vernier caliper	-	Patch thickness Studies
3.	Electronic balance	Sortorius, Germany	Weighing purpose
4.	Digital pH meter	Elico Ltd, AP, India	Surface pH study
5.	Fourier transform Infrared Spectrometer	Perkin-Elmer, USA	Compatibility studies
6.	Magnetic Stirrer	ROTEK, W.Vengola, Kerala, India	Diffusion Studies
7	UV Spectrophotometer	Shimadzu 1700, Japan	Determination of Absorption maxima & concentration of active substances

6.3. Preformulation Studies

Preformulation testing is the first step in the rational development of dosage forms of drugs substance. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms that can be man produced.
The following Preformulation studies are carried out

- Finding the absorption maxima
- Physical appearance
- Solubility
- Standard curve
- Infrared spectroscopy studies (compatibility studies)

6.4. Finding the absorption maxima (λ max)

The absorption maxima was found for identification of drug. Ultraviolet Visible spectrophotometry has been used to obtain specific information on the chromophoric part of the molecules. Organic molecules in solutions when exposed to light in the Visible/ Ultraviolet region of the spectrum absorb light of particular wavelength depending on the type of electronic transition associated with the absorption.

The extract solution (5, 10, 15, 20, 25 µg/ml) in distilled water was taken in a standard cuvette and scanned in the range of 200-800 nm in UV spectrophotometer. It exhibits maxima at 382 nm. Therefore, further all measurements were taken at 382 nm.

6.5. Collection of *Azadirachta indica* A Juss. leaves

Azadirachta indica A Juss. leaves were collected from Periyar College of Pharmaceutical Sciences campus, Tiruchppalli, Tamilnadu. Collected leaves were authenticated by Botanist, Dept. of Botany, National College, Trichy. Leaves were cleaned and shade dried at room temperature.

6.6. Extraction of leaves of *Azadirachta indica* A Juss.[37]

The shade dried leaves were subjected to size reduction and passed in to sieve no 20 and then 40. About 500g of the dried powder was extracted continuously in Soxhlet apparatus with petroleum ether for 24 hrs to remove the waxy materials. Then it was extracted with distilled for 72h. After 72h, the water substance was evaporated to obtain the crude extract (7.4%w/v). The extract was dried under vacuum oven.

Fig No:19. Soxhylation

6.7. Phytochemical Studies [38,39]

The AE (Aqueous Extract) was subjected to phytochemical studies to find out the presence and absence of constituents.

Table No.3: Phytochemical Tests

EXPERIMENT	OBSERVATION	INFERENCE
Test for Alkaloids		
1.Dragendroff's test: The extract was treated withDragendroff's reagent (potassium bismuth iodide solution)	Orange brown Precipitate was formed	Presence of alkaloids
2.Mayers' reagent's: The extract was treated with Mayer's (potassium mercuric iodide solution) reagent	Precipitate formed	Presence of alkaloids
3.Wagner's reagent: The extract was treated with wagner's reagent (iodide and potassium triiodide solution)	Reddish brown Precipitate was formed	Presence of alkaloids
Test for Glycosides		
1.Brontragers test: To the extract add dilute H2SO4 and filtered. Filtrate was extract with little chloroform layer was separated out and add equal volume of dilute NH3.	Red colour observed in ammonical layer	Presence of glycosides
Test for Saponin glycosides		
1.Foam test: Shake the extract with water.	Foam was produced/formed	Presence of saponin Glycosides
Test for Tannins and Phenolic compounds		
1.Ferric chloride test: To the aqueous extract few drops of ferric chloride solution were added	Dark black colour formed	Presence of tannins and phenolic compounds
2.Bromine water test: To the aqueous extract is treated with bromine water	Discoloration of bromine Water	Presence of tannins and phenolic compounds
3.$KMnO_4$test: To the aqueous extract is treated with dilute KMnO4.	Discoloration of solution	Presence of tannins and phenolic compounds.

Test for Reducing sugar		
1.Benedict's test: 0.5ml of extract solution 1ml of water 5 to 8 drops of fehlings solution was added.	No brick red precipitate	Absence of reducing sugar
Test for Amino acids		
1.Ninhydrin test: The aqueous extract is heated with 5%ninhydrin solution on boiling water bath for 10 min.	No purple colour formed	Presence of amino acids
2.The aqueous extract is treated with solution sodium hydroxide and lead acetate solution and boiled	No black precipitate is formed	Presence of amino acids
Test for Flavonoids		
1.Shinoda test: To the methanol extract add potassium hydroxide solution and then 10% ammonia.	Yellow colour Precipitate formed.	Presence of flavonoids
2.To the ethanol extract, add few drops of Lead acetate solution.	Yellow colour Precipitate formed.	Presence of flavonoids.
Test for Terpenoids		
1.4gm of extract was treated with 0.5ml of acetic anyhydride and 0.5ml of chloroform and added concentrated solution of sulphuric acid	No Red violet colour was obtained	Absence of terpenoids
Test for Steroids		
1. Libermann- Buchard Test: To extract add chloroform solution a few drops of acetic anhydride and 1ml of con. H2SO4 were added through the side of the test tube and set aside for a while.	Brown ring was formed at the junction.	Presence of steroids

2. Salkowski Test: To the extract add chloroform solution few drops of con. H_2SO_4 was added shaken and allowed to stand.	Greenish fluorescence was formed.	Presence of steroids
3. Libermann's Reaction: Mix 3ml of extract with 3ml of acetic anhydride, heat and cool. Add few drops of Con. H_2SO_4.	Blue colour was formed	Presence of steroids

6. 8. Preparation of Transdermal Patch [40]

Six batches of Aqueous Extract of leaves of *Azadirachta indica* A. Juss. transdermal patches were prepared using drug with two different polymer in three different ratio (1:4,1:6 &1:8). Weighed quantity of polymer was dissolved in calculated quantity of water and heated on a water bath. Calculated amount of extract was added to the above mixture and stirred well until a homogenous mixture was formed. Then calculated amount of permeation enhancer and glycerin were added. In all the six batches the quantity of extract was same.

The resultant mixture was poured into a Petridish and air dried at room temperature for 24h.The patches were then peeled off from the Petridish with the help of a knife and kept in desiccator.

Table No.4: Formula for TDDS

Ingredients	**Formulation Code**					
	TP1	**TP2**	**TP3**	**TS4**	**TS5**	**TS6**
AE (mg)	40	40	40	40	40	40
Pectin (mg)	160	240	320	-	-	
S.Alginate (mg)	-	-	-	160	240	320
DMSO (ml)	0.3	0.3	0.3	0.3	0.3	0.3
Glycerin (ml)	0.3	0.3	0.3	0.3	0.3	0.3
Water	q.s	q.s	q.s	q.s	q.s	q.s

Calculation

Diameter of glass plate = X cm

Radius of glass plate = Y cm

Area of glass plate = $\pi r^2 (\pi=3.14)$

= 3.14 × (y)

= Z cm2

Z/X= Area/diameter = capacity (ml)

6.9. Preparation of Calibration curve of *Azadirachta indica* A. Juss. Extract

Accurately weighed quantity (100mg) of AE was transferred into a 100ml volumetric flask and dissolved in small amount of distilled water (D.W) and made up to the volume to make the standard stock solution of 1 mg/ml.

From the stock, 1ml was taken in 10ml volumetric flask and made up the volume with the buffer; from this solution 0.5ml to 3ml solution was transferred to 10ml volumetric flask and made up to required volume with more D.W and the resulting concentration ranges from 5 to 50 µg/ml. The absorbance of these solutions was determined at 382nm using UV spectrophotometer. The calibration curve was constructed between the absorbance and concentration.

6.10. Preparation of phosphate buffer pH 7.4

Phosphate buffer pH 7.4 was prepared as per the method described in I.P 1996 using disodium hydrogen phosphate and sodium hydroxide. The pH was adjusted to 7.4 prior to quantitative estimation.

6.11. Physico chemical evaluation of *Azadirachta indica* A.Juss. Transdermal Patch [41,42]

Formulated patches were subjected to the preliminary evaluation tests. Patches with any imperfections, entrapped air, or differing in thickness, weight (or) content uniformity were excluded from further studies.

1. **Uniformity of weight**

This was done by weighing five different patches of individual batch taking the uniform size at random and calculating the average weight of three. The tests were performed on patch which was dried at 60˚C for 4 h prior to testing.

2. Thickness of the Patch

The thickness of the patch was assessed by using digital vernier caliper at different points of the patch. From each formulation three randomly selected patches were used. The average value for thickness of a single patch was determined.

3. Drug content determination

The patches were taken and added to a beaker containing 100 ml of D.W. The medium was stirred magnetic bead for 5 h. The solution was later filtered and analyzed for drug content with proper dilution at 382 nm spectrophotometrically.

4. Folding Endurance

This was determined by repeatedly folding one patch at the same place till it broke. The number of times the patch could be folded at the same place without breaking gave the value of folding endurance.

5. Percentage Moisture uptake

The patch were weighed accurately and placed in desiccators containing aluminium chloride. After 24 h, the patch were taken out and weighed. The percentage moisture uptake was calculated as the difference between final and initial weight. With respect to initial weight. It is calculated by using following formula.

$$\text{Percentage moisture content} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

6. Percentage Moisture content

The patch were weighed and kept in desiccators containing calcium chloride. After 24h the patch were taken out and weighed. The percentage moisture content was calculated using the following formula.

$$\text{Percentage moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

7. Determination of surface pH

The patches were allowed to swell by keeping them in contact with 1 ml of distilled water for 2 h at room temperature and pH was noted down by bringing the electrode in contact with the surface of the patch, allowing it to equilibrate for 1 min.

8. Percent Elongation

When stress is applied, a patch sample stretches and this is referred to as strain. Strain is basically the deformation of patch divided by original dimension of the sample. Generally elongation of patch increases as the plasticizer content increases. It is calculated by using following formula.

$$\text{Percentage elongation} = \frac{\text{Increase in length of patch}}{\text{Initial length of patch}} \text{ X } 100$$

9. Tensile strength

Tensile strength is the maximum stress applied to a point at which the patch specimen breaks. It is calculated by the applied load at rupture divided by the cross-sectional area of the strip as given in the equation below

$$\text{Percentage elongation} = \frac{\text{Load at failure}}{\text{Patch thickness x Patch width}} \text{ X } 100$$

The results of Physico chemical parameters are shown in Table:14

Cellophane Membrane Treatment

Cellophane membrane was boiled in the D.W for 1 h and washed with fresh D.W for three times and kept in ethanol for 24 h. It was washed with D.W and treated with 0.3% sodium sulphite and soaked in distilled water for 2 min at 60°C followed by acidified with 0.2% sulphuric acid. Finally the membrane was dipped in boric buffer (pH 9) till it is used for permeation study.

Drug Permeation Studies [43]

The *in vitro* diffusion rate of *Azadirachta indica* A. Juss. transdermal patches were evaluated by open ended tube through using distilled water as diffusion medium up to 8 h studies. The cellophane membrane was tide in one end of the tube and then immersed in the receptor compartment containing 200ml of 7.4 buffer solution. Which was stirred at medium speed and maintained at 37°C±2°C. Samples were withdrawn at regular time intervals and the same volume was replaced by fresh diffusion medium. The samples were analyzed using UV – visible spectrophotometer (Shimadzu UV1700) set at 382 nm.

The results are shown in Table 25 and Fig No 33

6.12. Experimental conditions of *ex vivo* transdermal permeation study of P2 formulation[44]

Ex vivo Transdermal permeation studies carried out using Goat abdomen skin

- ✓ The receptor compartment consisted of 400ml of Phosphate buffer (pH 7.4) in 500 ml beaker
- ✓ Temperature was maintained at 37 ± 0.5°C and stirred at 900 rpm
- ✓ The *Azadiractha indica* A Juss. Transdermal patch was placed in Goat abdomen skin and tied to the one end of open-ended glass cylinder that was then dipped into freshly prepared phosphate buffer on magnetic stirrer
- ✓ Samples were taken from receptor medium at 0, 30, 60, 90, 120, 150, 180, 210, 240 and 270, 300, 330,360 , 390, 420, 660, 720 min
- ✓ Periodically 5ml of sample was withdrawn and same volume of medium was replaced with fresh buffer
- ✓ All the Samples were assayed spectrophotometrically at 382 nm using PB 7.4 pH as blank

The result shown in Table:31 and Fig No52

6.13. Diffusion Kinetics [45]

Data obtained from *in-vitro* diffusion studies were fitted to various kinetic equations. The kinetic models used are zero order equations (Q=k0t), First order equation {ln (100 – Q) =ln Q – klt}, Higuchi equation (Q=kt1/2), Hixson and crowell model Qt1/3 Vs t and Qt2/3 Vs – Modified root cube equation. Further, to find out the mechanism of drug diffusion, first 60% drug diffusion was fitted in Korsmeyer and Peppas equation (Q=kptn). Where, Q is the percent of the drug diffusion at time t and k0 and kt are the coefficients of the equations and ‘n’ are the diffusion exponent. The ‘n’ value is used to characterize different diffusion mechanism.

The order of drug diffusion can be assessed by graphical treatment of drug diffusion data.

A plot of cumulative % drug diffusion versus time would be linear if the drug diffusion follows zero order (i.e. Concentration independent diffusion).

A plot of log of % remaining drug versus time would be linear, if the drug diffusion follows first order (i.e. Concentration dependent diffusion)

The linear equation for zero order drug diffusion plot is:

$$Ct = C0 - Kt$$

Where,

Ct = concentration remaining at time t,

Co = original concentration,

t = time,

K = diffusion rate

The linear equation for first order diffusion plot is

$$\text{Log } C = \frac{\log C0 - Kt}{2.303}$$

A matrix device as the name implies, consists of drug dispersed homogeneously throughout a polymer matrix

In this model, drug in the outside layer exposed to the bathing solution is dissolved first and then diffuses out of the matrix. This process continues with the interface between the bathing solution and the solid drug moving towards the interior. Obviously, for this system to be diffusion controlled, the rate of dissolution of drug particles within the matrix must be much faster than the diffusion rate of dissolved drug leaving the matrix.

Hydrophilic matrix tablets contain a water swellable polymer. On

$$[1 - Mt / M] 1/3 = 1\text{-}kt$$

Where,

Mt = mass of drug diffusion at time t,

M = mass diffusion at the infinite time,

K = rate of erosion,

t = time

Thus a plot of [1 – Mt / M] 1/3 versus the time will be linear. If the diffusion of drug from the matrix is erosion controlled.

In order to ascertain whether the drug diffusion occurs by diffusion or erosion, the drug diffusion data was subjected to following modes of data treatments.

1) Amount of drug diffusion versus square root of time (Higuchi Plot).

2) [1 – Mt / M] 1/3 versus time.

6.14.Stability studies [46,47]

a) Stability

Stability is official defined as the time lapse during which the drug product retains the same property and characteristics that it possessed at the time of manufacture. This process being early development phases.

Instability in modern formulation is often undetectable only after considerable storage period under normal conditions. To assess the stability of a formulated product it is usual to expose it to high stress conditions to enhance deterioration and therefore the time required for testing is reduced. Common high stresses are temperature and humidity. This will eliminate unsatisfactory formulation.

b) Strategy of stability testing

- The study of drug decomposition kinetics
- The development of stable dosage form
- Establishment of expiry date for commercially available drug product is some of the needs of stability testing
- Data from which study should be provided on at least 3 primary batches of the drug product
- The batches should be manufactured to a minimum of pilot scale
- Important point of view of the safety of the patient, patient relieves a uniform dose of drug throughout the shelf life of the product

Table No.5: Stability condition chart

Intended Storage Condition	**StabilityTest Method**	**ICH Test Temperature and Humidity (period in months)**	**WHO Test Temperature and Humidity(period in Months)**
Room Temperature	Long term	25±20C/60±5%RH	25±20C/60±5%RH or 30±20C/65±5%RH 30±20C/75±5%RH
	Intermediate	30±20C/65±5%RH	30±20C/65±5%RH
	Accelerated	40±20C/75±5%RH	40±20C/75±5%RH
Refrigerated	Long term Accelerated	50C/ambient 25±20C/60±5%RH	5±30C 25±20C/60±5%RH or 30±20C/65±5%RH
Freezer	Long term	-200C/ambient	-200C±50C

6.15. Screening of Antimicrobial activity of *Azadirachta indica* A.Juss. [48,49,50]

6.15.1. Anti-Bacterial Activity

Principle

Discs impregnated with known concentration of antibiotics discs are placed on agar plate that has been inoculated (or) seeded uniformly over the entire plate with a culture of the bacterium to be tested. The plate is incubated for 18-24 hrs at 37oC. During this period, the antibacterial agent diffuses through the agar and may prevent the growth of organism. Effectiveness of susceptibility is proportional to the diameter of inhibition of zone around the disc. Organisms which grow up to the edge of the disc are resistant.

A) **Materials Required**

Peptone, Sodium Chloride, Dil. Sodium Hydroxide, Dil. Sulphuric acid, Agar, Distilled water, pH paper, Conical flask, Culture tubes, Glass rod, Non-absorbant cotton, Autoclave- Micropipette, Petri dishes and Incubator.

B) Experimental condition

Organisms used	**:**	*Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa*
Media used	**:**	Nutrient Agar.
Test used	**:**	AE patch
Standard	**:**	Ciprofloxacin, Nystatin

C) Preparation of Nutrient Agar

8.2 gm of agar powder was dissolved in 250 ml of water. The medium was steamed in boiler to precipitate any heat coagulable material. Then the medium was filtered. The filtrate was distributed in 5ml quantity in to culture tubes. The tubes were plugged with non-absorbent cotton. The medium in the tubes were sterilized by autoclave not less than 15 minutes at 15 pounds per sq. inch at 121^{o}C.

D) Preparation of Paper Disc

By using standard punching machine what man filter paper was cut and standard paper of 6.0 mm diameter was prepared. The paper discs were sterilized in a hot air oven at 160^{o}C for 1hour. The paper discs were then impregnated with the test solution.

6.15.2. Anti-Fungal Activity

Principle

Discs impregnated with known concentration of antibiotics discs are placed on Modified Sabouraud's Glucose agar plate that has been inoculated (or) seeded uniformly over the entire plate with a culture of the fungi to be tested. The plate is incubated for 3 days 18-24 hrs at 37^{o}C. During this period, the antifungal agent diffuses through the agar and may prevent the growth of organism. Effectiveness of susceptibility is proportional to the diameter of inhibition of zone around the disc. Organisms which grow up to the edge of the disc are resistant.

A) Materials Required

Glucose, Peptone, yeast extract, Glycerin monostearate, Olive oil, Tween 80, Chloramphenicol, Agar, Distilled water, pH paper, Conical flask, Culture tubes, Glass rod, Non-absorbant cotton, Autoclave, Micropipette Petri dishes and Incubator.

B) Experimental condition

Organisms used	**:**	*Candida albicans, Aspergillus niger.*
Media used	**:**	Modified Sabouraud's Glucose Agar medium
Test used	**:**	AE patch,
Standard	**:**	Ciprofloxacin, Nystatin

7. RESULTS AND DISCUSSIONS

7.1. Absorption maxima (λ max) of Aqueous Extract of *Azadirachta indica* A. Juss

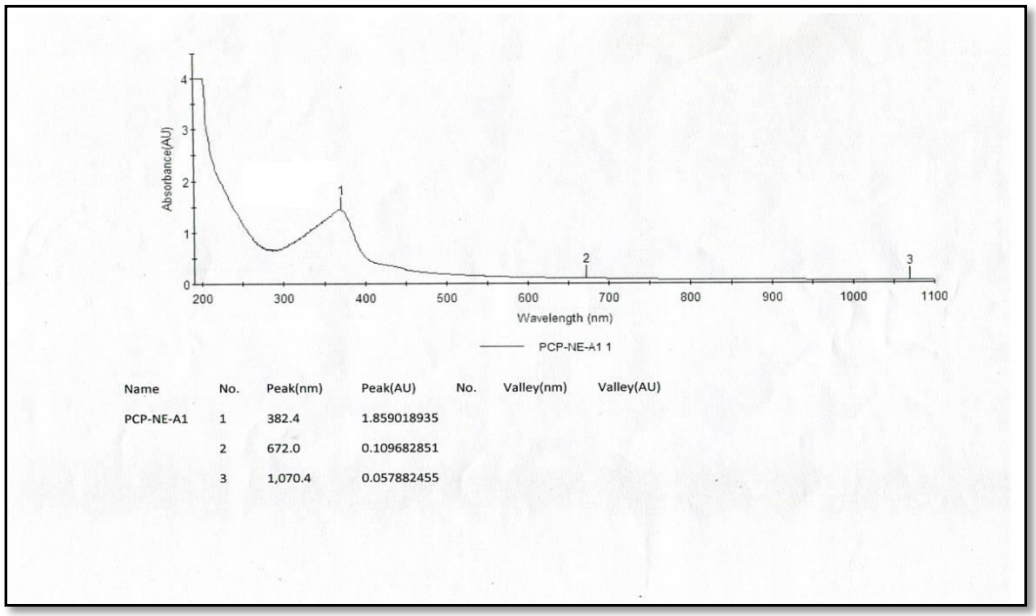


Fig No.20: Absorption maxima (λ max) of Aqueous Extract of *Azadiractha indica* A Juss.

The sharp peak observed at 382 nm, further measurements were taken at 382nm.

7.1.1. Standard Curve values of Aqueous Extract of *Azadirachta indica* A. Juss

Table No.6: Standard values of Aqueous Extract of *Azadirachta indica* A. Juss

Concentration (μg/ml)	**Absorbance at 382 nm**
	Average ± SD
0	0.000± 0.000
10	0.091± 0.001
20	0.182± 0.001
30	0.273± 0.001
40	0.363± 0.001
50	0.432± 0.001
60	0.512±0.008

Mean ± S.D: n = 3

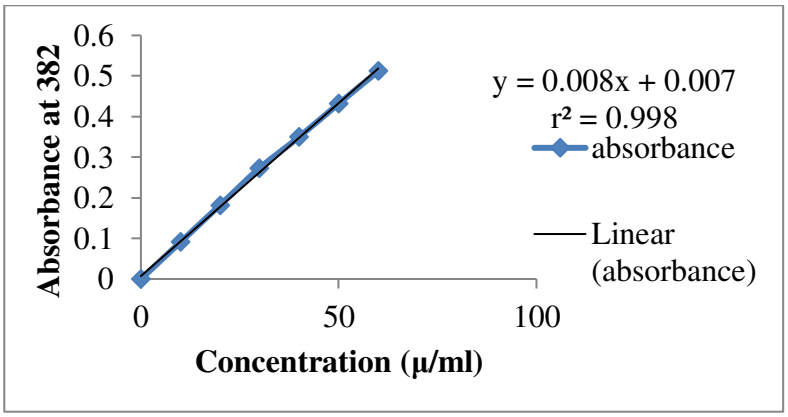


Fig No.21: Standard Curve of Aqueous Extract of *Azadirachta indica* A.Juss

7.2. Physical appearance

Colour	:	Green
Taste	:	Bitter taste
Solubility	:	Freely soluble in Distilled Water

7.3. Phytochemical Studies

Table No.7: Phytochemical constituents

S.No.	Chemical constituents	Aqueous Extract
1.	Alkaloids	+
2.	Saponins	+
3.	Tannins	+
4.	Phenolic	+
5.	compounds	+
6.	Flavonoids	+
7.	Steroids	+
8.	Glycosides	+
9.	Amino acids	-
10.	Reducing sugar	-
	Terpenoids	

(+) Presence of constituents (-) Absence of constituents

The phytochemical studies revealed that the presence of alkaloids, saponins, tannins, phenolic compounds, flavonoids and sterol.

7.4. Hygroscopic Nature

Table No. 8. Hygroscopic Nature determination

At Room Temperature	75%RH at 40^0C
Sample No-1	Sample No-1
Weight gain observed (0.41±2.1)	Weight gain observed (0.38±0.6)

The results, it is observed that the prepared Transdermal patch is hygroscopic in nature.

7.5. Compatibility study

FTIR studies

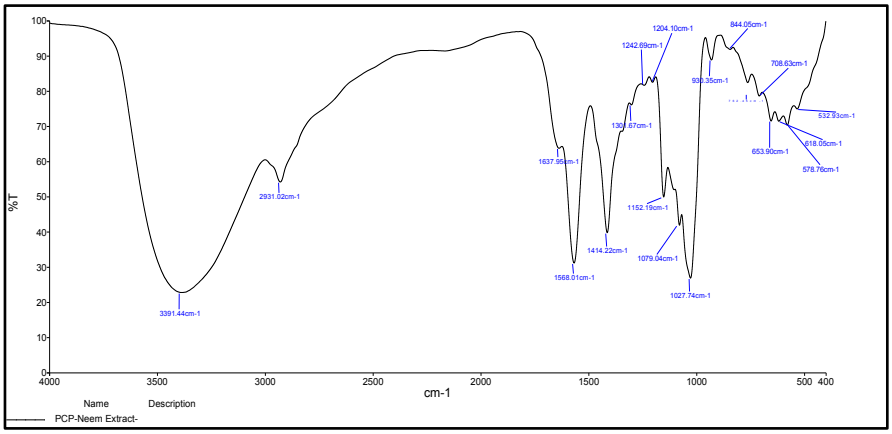


Fig No. 22: FTIR Spectrum of Aqueous Extract of *Azadirachta indica* A. Juss

Table No. 9. FTIR Interpretation of Aqueous Extract of *Azadirachta indica* A. Juss

Wave number (cm^{-1})	Functional Group
3391.615	C=O stretching
2931.66	C-H Stretching
1637.95	C-C Stretching
1414.22	OH Bending
921.39	C-O Stretching
763.74, 653.03	C-H Rocking

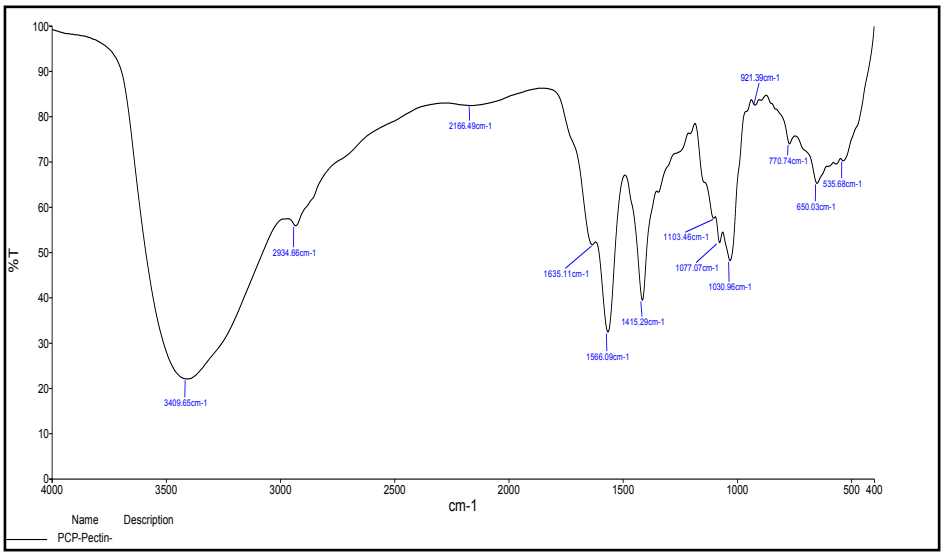


Fig No. 23: FTIR spectrum of Pectin

Table No.10: FTIR Interpretation of Pectin

Wave number (cm-1)	Functional groups
3409.65	C=O stretching
2934.66	C-C stretching
1635.11	C-C stretching
1415.29	OH bending
921.39	C-O Stretching
770.76	CH Rocking

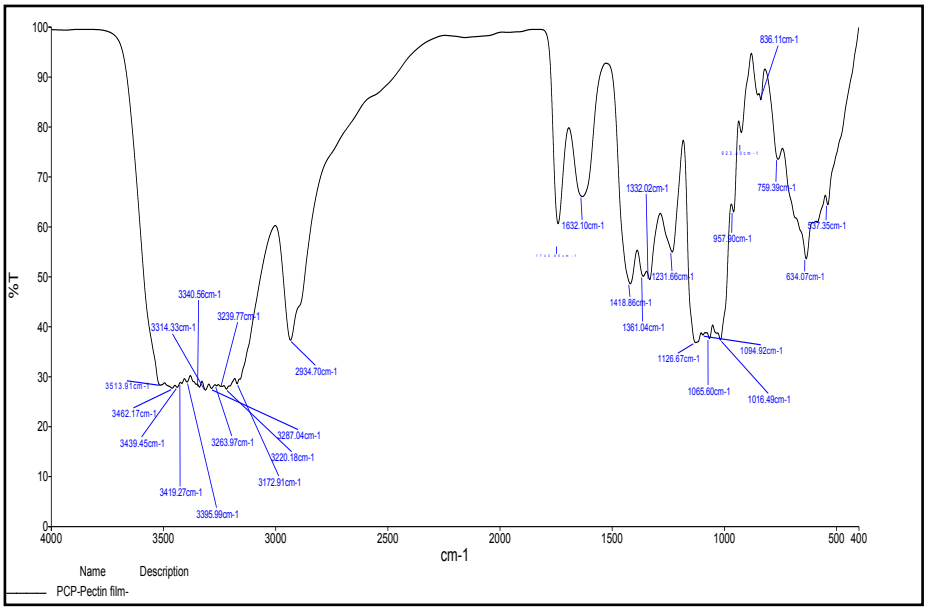


Fig No. 24: FTIR spectrum of Pectin formulation

Table No.11: FTIR Interpretation of Pectin formulation

Wave number (cm-1)	Functional groups
3462.17	C=O stretching
1740.66	C-C stretching
1632.11	C-C stretching
1126	OH bending
923.100	C-O Stretching
759.39	CH Rocking

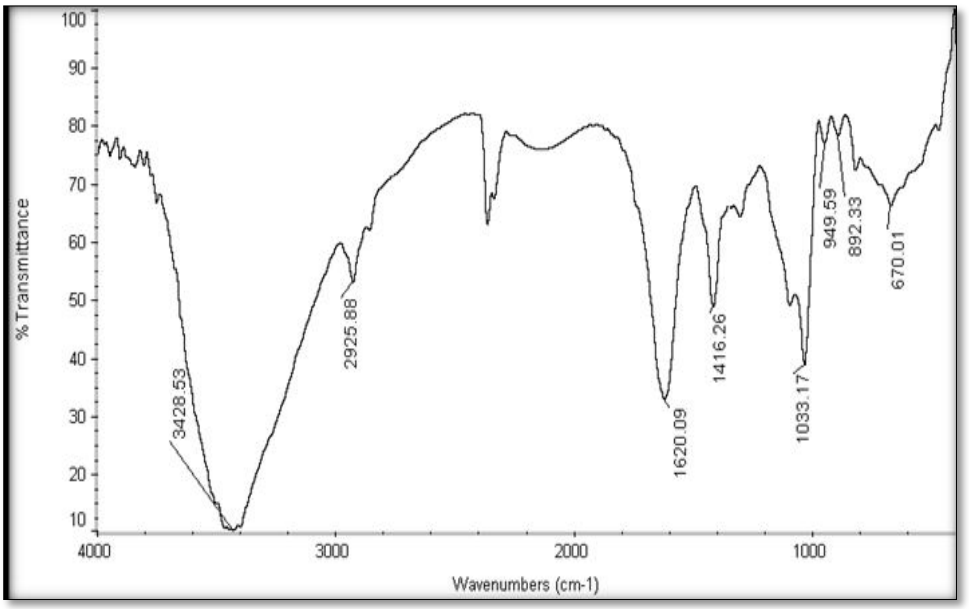


Fig.No. 25: FTIR Spectrum of Sodium alginate

Table No. 12: FTIR Interpretation of sodium alginate

	Wave number (cm^{-1})	**Functional groups**
1	3428.58	C-H stretching
2	2925.88	C-H stretching
3	1620.09,1416.26	C-C multiple bond stretching
4	1416.26	O-H stretching

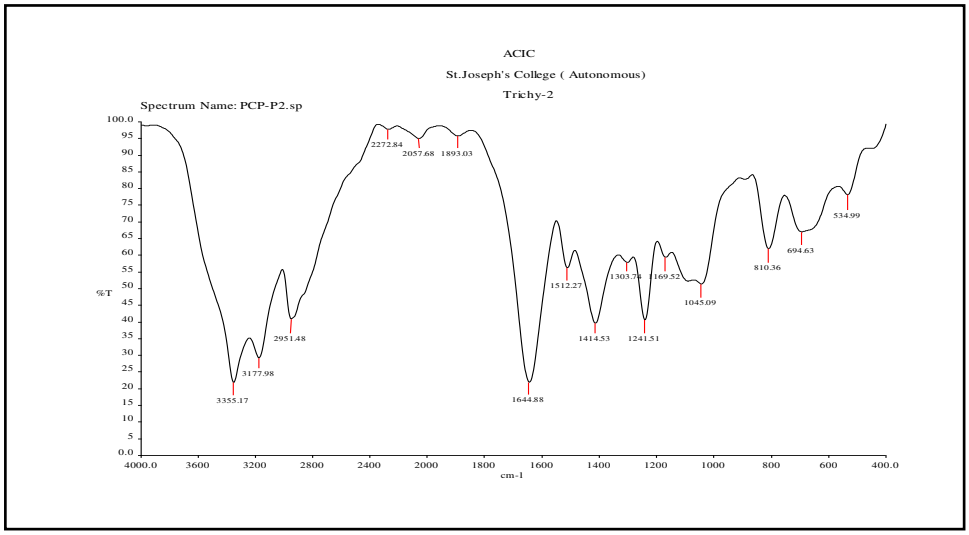


Fig No. 26: FTIR Spectrum of Sodium alginate formulation

Table No.13: FTIR Interpretation of Sodium alginate formulation

Wave number (Cm^{-1})	Functional groups
3355.17	O-H stretching
1414.53	O-H bending
1045.91	C-O stretching
1115.29	C-O stretching
1045.09	C-O stretching
810.36	C-H (Out plane bending)
694.63	C-H (Out plane bending)

The FTIR graphs of drug, excipients and formulations results showed that there is no extra peak (or) broadening of peaks were observed and thus it indicates that there is no incompatibility between drug and excipients.

7.6. Physico chemical evaluation of Aqueous Extract of *Azadirachta indica* A. Juss Transdermal patch

Table No.14: Physico chemical evaluation of Aqueous Extract of *Azadirachta indica* A. Juss Transdermal patches

Formulation code		Uniformity of weight (g)	Thickness (mm)	Drug content (%)	Folding Endurance e(no's)	Moisture Uptake (%)	Moisture Content (%)	Surface pH	Percent Elongation (% mm)	Tensile Strength (Kg/mm^2)
Transdermal Patch of Pectin (P)	P1	0.38±0.14	0.43±0.73	85.23±0.92	235±0.76	2.14±0.08	2.866±0.07	7.1±0.10	85±0.32	5.300±0.55
	P2	**0.46±0.85**	**0.49±0.23**	**85.69±0.56**	**249±0.23**	**2.85±1.03**	**3.422±0.22**	**7.3±0.72**	**91±1.11**	**6.361±0.87**
	P3	0.49±0.10	0.50±1.13	88.98±0.16	254±0.36	3.79±1.03	3.940±0.36	7.2±0.72	86±1.08	6.7031±0.87
Transdermal Patch of Sodium alginate (S)	S4	0.39±0.43	0.36±0.33	82.03±0.22	241±0.72	2.75±0.65	1.736±0.46	7.0±0.33	82±0.23	5.513±0.86
	S5	**0.41±0.65**	**0.45±0.76**	**83.35±0.94**	**265±0.44**	**1.97±0.44**	**1.657±0.03**	**7.1±0.12**	**87±0.92**	**6.253±0.62**
	S6	0.48±0.50	0.47±0.16	80.13±0.40	247±0.42	2.79±0.35	2.457±0.03	7.2±0.12	89±0.86	6.432±0.74

Mean ± S.D: n = 3

The six (P1, P2,P3,S4 S5, S6) batches of extract loaded patches with different ratios of two different polymers were subjected to various physicochemical evaluations.

Based on thickness, uniformity of weight, folding endurance, percentage moisture uptake moisture content and tensile strength, the formulations P2 and S5 were selected for further studies.

7.7. Optimized formula of *Azadirachta indica* A Juss. Transdermal patch

Table No.15: Optimized formula of *Azadirachta indica* A Juss. Transdermal patch

S.NO.	Ingredients	P (Pectin)	S (Sodium alginate)
1.	Extract (mg) *Azadirachta indica*	40	40
2.	Polymer (mg)	240	240
3.	DMSO/SLS(ml)	0.3	0.3
4.	Glycerin (ml)	0.3	0.3
5.	Water (ml)	q.s	q.s

7.8.Pictures of Prepared Patches

Fig No. 27: Transdermal Patch of P1

Fig No. 28: Transdermal Patch of P2

Fig No. 29: Transdermal Patch of P3

Fig No.30: Transdermal Patch of S4

Fig No. 31: Transdermal Patch of S5

Fig No. 32: Transdermal Patch of S6

7.9. Physicochemical Evaluation of Selected formulations

Table No. 16: Uniformity of weight

S. No.	Formulation code	Weight(g)
1.	**Pectin (P2)**	**0.46±0.85**
2.	Sodium alginate (S5)	0.41±0.65

Mean ± S.D: n= 3

Table No. 17: Thickness of the patch

S. No.	Formulation code	Thickness (mm)
1.	**Pectin (P2)**	**0.49±0.23**
2.	Sodium alginate (S5)	0.45±0.76

Mean± S.D:n=3

Table No. 18: Determination of Drug content

S. No.	Formulation code	% Drug content
1.	**Pectin (P2)**	**85.69±0.56**
2.	Sodium alginate (S5)	83.35±0.94

Mean ± S.D: n=3

Table No. 19: Folding Endurance

S. No.	Formulation code	Folding Endurance
1.	**Pectin (P2)**	**249±0.23**
2.	Sodium alginate (S5)	245±0.44

Mean ± S.D: n =3

Table No. 20: Percentage Moisture Uptake

S. No.	Formulation code	% Moisture uptake
1.	**Pectin (P2)**	**2.85±1.03**
2.	Sodium alginate (S5)	1.97±0.44

Table No. 21: Percentage Moisture Content

S. No.	Formulation code	% Moisture content
1.	**Pectin (P2)**	**3.422±0.22**
2.	Sodium alginate (S5)	1.657±0.03

Mean ± S.D: n=3

Table No. 22: Surface pH

S. No.	Formulation code	Surface pH of patches
1.	**Pectin(P2)**	**7.3±0.72**
2.	Sodium alginate(S5)	7.1±0.12

Mean ± S.D: n=3

Table No. 23: Percent Elongation

S. No.	Formulation code	% Elongation (%mm)
1.	**Pectin(P2)**	**91±1.11**
2.	Sodium alginate(S5)	87±0.92

Mean ± S.D: n=3

Table No.24: Tensile Strength

S. No.	Formulation code	Tensile strength (kg/mm^2)
1.	**Pectin(P2)**	**6.361±0.65**
2.	Sodium alginate(S5)	6.253±0.25

Mean ± S.D: n=3

7.10. *In vitro* drug difuusion study

Table No.25: *In vitro* drug diffusion study

Time in (min)	% drug diffusion					
	Pectin (P)			Sodium Alginate(S)		
	P1	P2	P3	S4	S5	S6
0	0	**0**	0	0	**0**	0
30	6.78	**5.2**	2.98	7.15	**4.86**	3.46
60	10.30	**8.6**	4.78	12.36	**9.5**	5.72
90	13.65	**11.21**	5.64	16.19	**11.98**	9.34
120	17.89	**15.96**	7.15	20.72	**16.28**	12.20
150	23.10	**18.54**	9.45	24.37	**20.41**	17.40
180	29.54	**22.34**	14.82	30.97	**25.01**	22.19
210	33.68	**27.45**	19.38	34.65	**32.21**	25.30
240	39.70	**31.23**	25.48	38.98	**36.43**	29.68
270	45.58	**38.42**	30.98	43.12	**42.11**	33.76
300	48.77	**43.58**	38.73	48.94	**46.36**	36.15
330	53.22	**49.86**	42.54	54.30	**52.21**	42.56
360	57.10	**55.25**	45.24	59.61	**55.34**	47.26
390	62.41	**60.46**	49.64	65.73	**59.43**	53.90
420	67.38	**65.46**	53.28	68.60	**64.22**	58.76
450	72.34	**69.46**	59.74	70.12	**68.18**	63.12
480	77.10	**73.04**	64.10	74.62	**71.46**	67.92

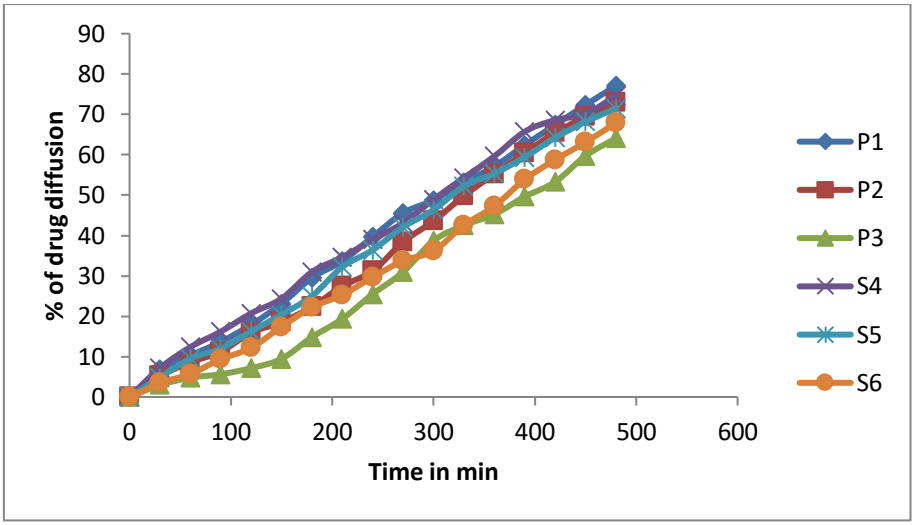


Fig No.33:*In vitro* drug diffusion study

Table No. 26: *In vitro* drug diffusion profile of P2

S. No.	Time (min)	% Drug diffusion of P2
1.	0	0
2.	30	5.2± 0.23
3.	60	8.6± 0.45
4.	90	11.21± 0.44
5.	120	15.96± 0.76
6.	150	18.54± 1.78
7.	180	22.34± 0.59
8.	210	27.45± 1.23
9.	240	31.23 ± 0.61
10.	270	38.42±1.24
11.	300	43.58± 1.90
12.	330	49.86±1.54
13.	360	55.25± 0.23
14.	390	60.46± 1.34
15.	420	65.46± 0.91
16.	450	69.46± 0.72
17.	480	73.04± 0.61

Mean±S.D:n=3

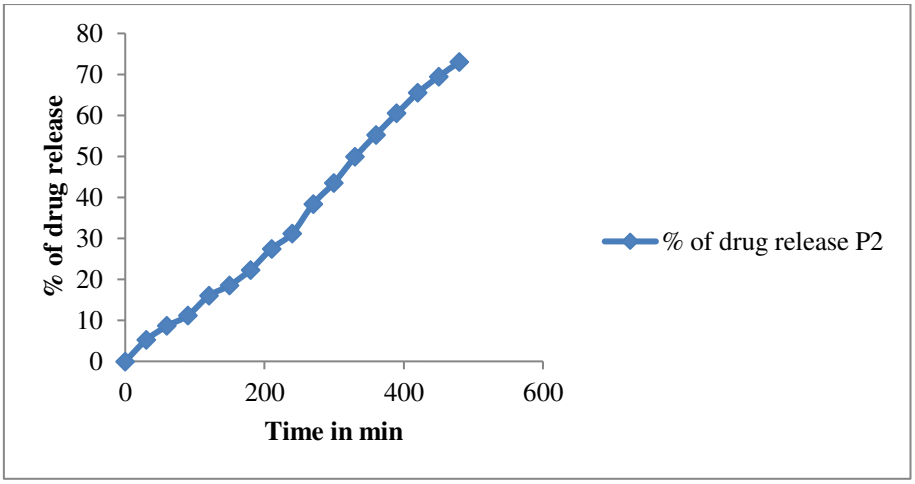


Fig No.34: *In vitro* drug diffusion profile of P2

Table No. 27 : *In vitro* drug diffusion profile of S5

S.No	Time (min)	% Drug diffusion of S5
1	0	0
2	30	4.86± 0.23
3	60	9.95± 0.45
4	90	11.98± 0.44
5	120	16.28± 0.76
6	150	20.41± 1.78
7	180	25.01± 0.59
8	210	32.21± 1.23
9	240	31.23 ± 0.61
10	270	42.11±1.24
11	300	46.36± 1.90
12	330	52.21±1.54
13	360	55.34± 0.23
14	390	59.43± 1.34
15	420	64.22± 0.91
16	450	68.18± 0.72
17	480	71.46± 0.61

Mean S.D:n=3

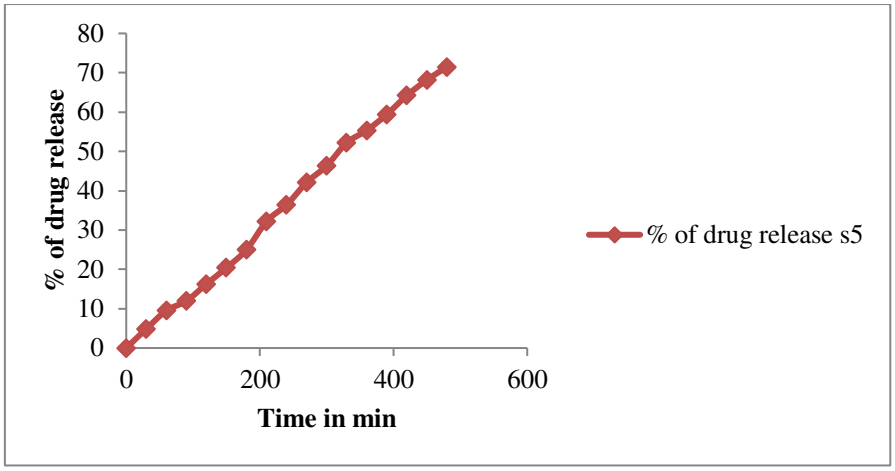


Fig No.35: *In vitro* drug diffusion profile of S5

Table No.28 : Comparative *in vitro* drug diffusion profile

S.No.	Time (min)	% of drug diffusion P2	% of drug diffusion S5
1	0	0	0
2	30	5.2± 0.23	4.86± 0.23
3	60	8.6± 0.45	9.95± 0.45
4	90	11.21± 0.44	11.98± 0.44
5	120	15.96± 0.76	16.28± 0.76
6	150	18.54± 1.78	20.41± 1.78
7	180	22.34± 0.59	25.01± 0.59
8	210	27.45± 1.23	32.21± 1.23
9	240	31.23 ± 0.61	31.23 ± 0.61
10	270	38.42±1.24	42.11±1.24
11	300	43.58± 1.90	46.36± 1.90
12	330	49.86±1.54	52.21±1.54
13	360	55.25± 0.23	55.34± 0.23
14	390	60.46± 1.34	59.43± 1.34
15	420	65.46± 0.91	64.22± 0.91
16	450	69.46± 0.72	68.18± 0.72
17	480	73.04± 0.61	71.46± 0.61

Mean S.D:n=3

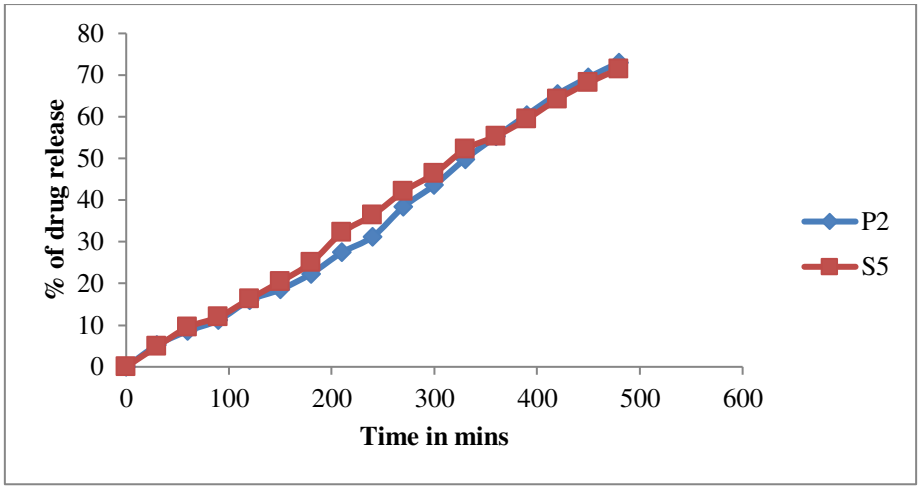


Fig No.36: Comparative *in vitro* drug diffusion profile

The selected 2 batches of formulations Pectin (P2), Sodium alginate (S5) were subjected comparative *in vitro* permeation studies. Formulation P2 showed sustained diffusion in a controlled manner upto 8 hrs.

7.11. Release Kinetics

Table No: 29. *In vitro* Release kinetics values

Formulation code	Correlation coefficient(r^2)				'n'- Diffusion Exponent
	Zero order	First order	Higuchi	Korsmeyer peppas	
P2	0.997	0.976	0.883	0.882	0.865
S5	0.991	0.945	0.997	0.853	0.853

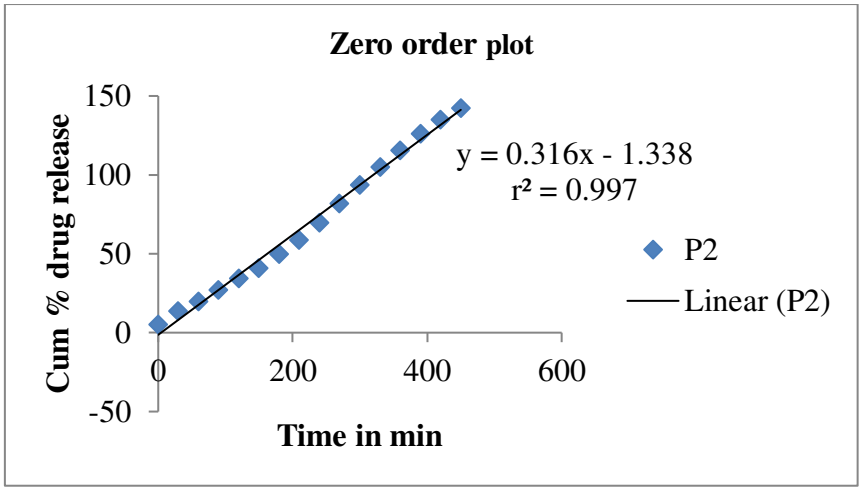


Fig No.37: Zero order kinetic plot of P2

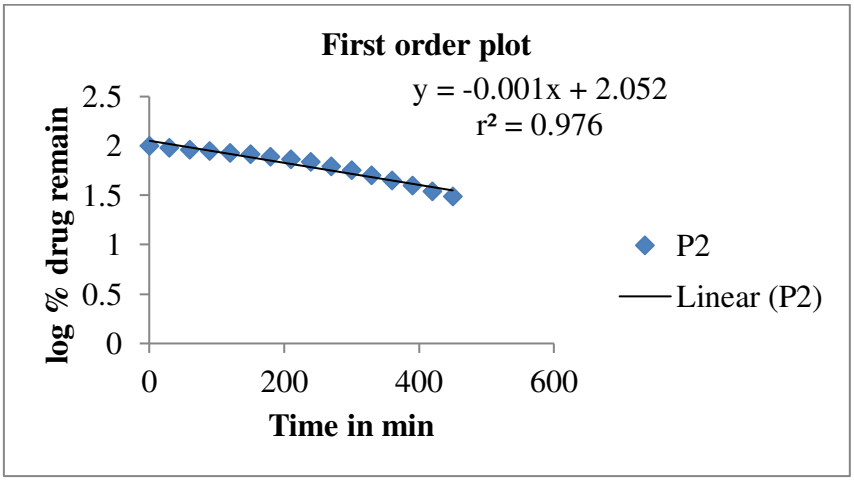


Fig No.38: First order kinetics plot of P2

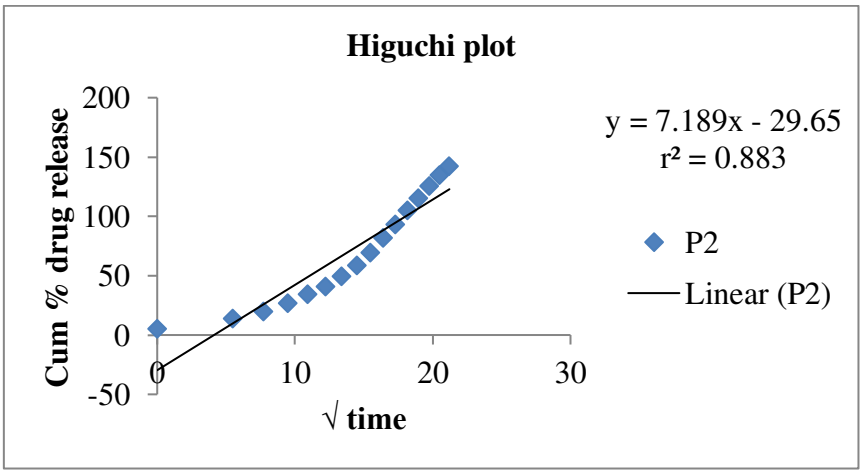


Fig No.39: Higuchi plot P2

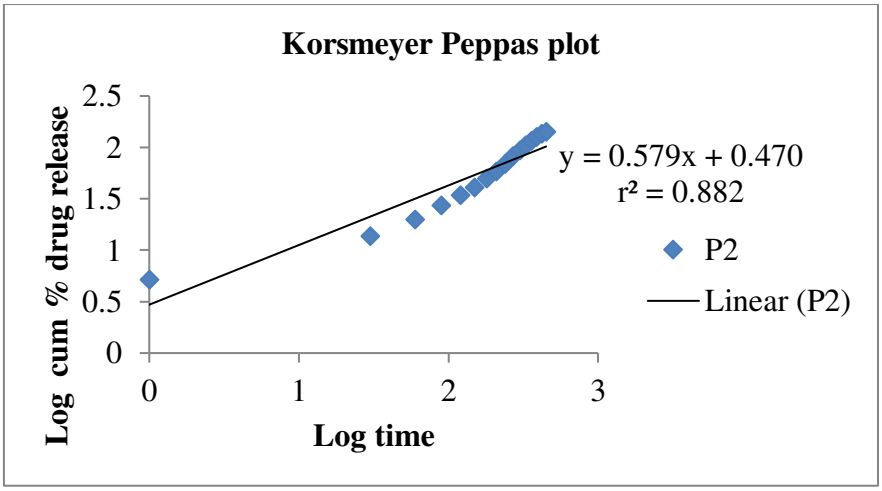


Fig No.40: Korsmeyer peppas plot of P2

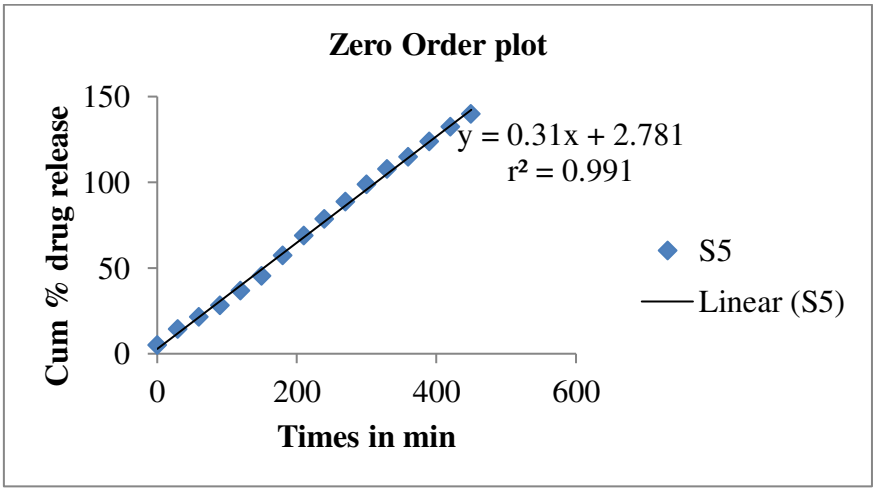


Fig No.41: Zero order plot of S5

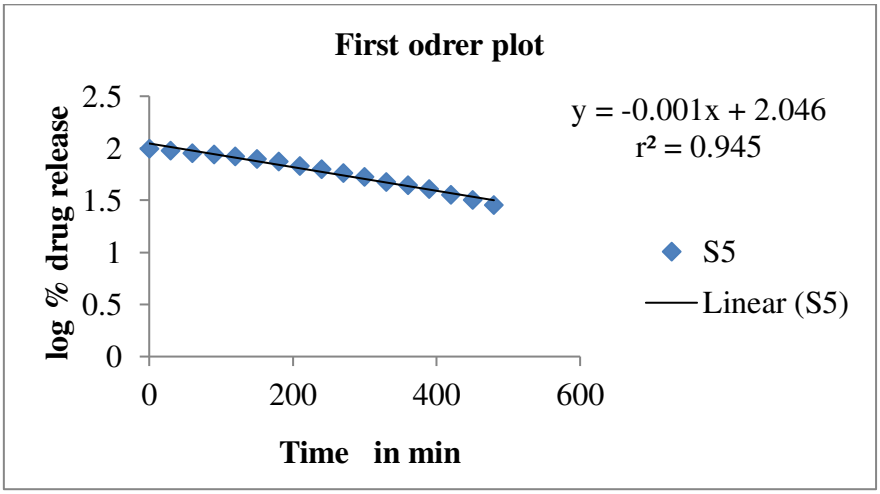


Fig No.42: First order plot of S5

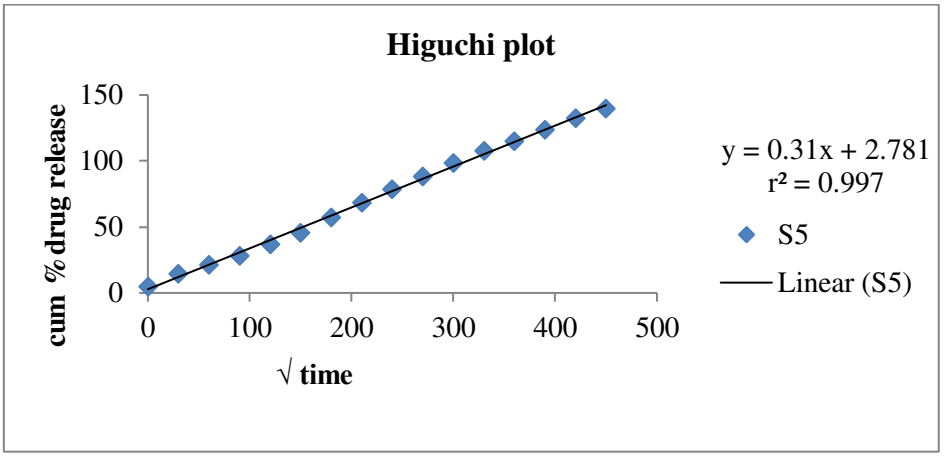


Fig No. 43: Higuchi plot of S5

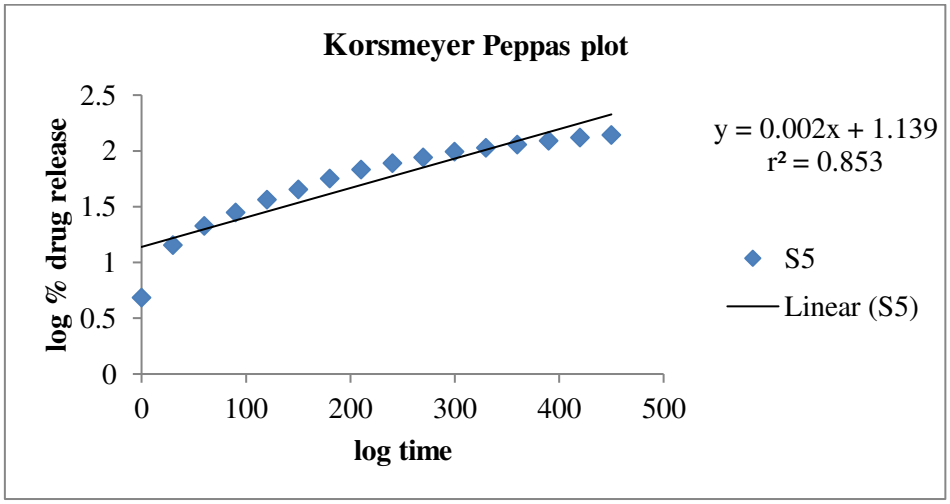


Fig No.44: Korsmeyer peppas plot of S5

Release kinetics study revealed that both (P2 & S5) selected patches follows zero order and non fickian diffusion model. So they were subjected to antimicriobial screening.

7.12. Screening of Antimicrobial activity of *Azadirachta indica A. juss*

The anti - microbial activity for the given sample was carried out by disc diffusion technique (Indian Pharmacopoeia 1996, vol II A-105). The test micro organism of *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia* and *Proteus vulgaries* and fungus *Aspergilus niger, Candida albicans* were obtained from National Chemical Laboratory (NCL) Pune and maintained by periodical sub culturing on nutrient agar and sabouraud dextrose agar medium for bacteria and fungi respectively. The effect produced by the sample was compared with the effect produced by the positive control (reference standard Ciprofloxacin 5µg/ disc for bacteria; Nystatin100µg/ disc for fungi).

For Fungi

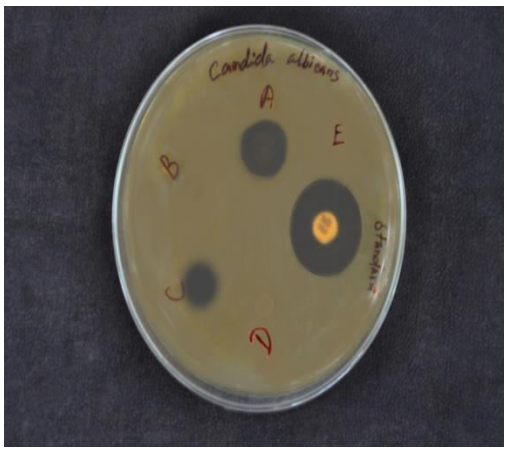
After 72h the plates were observed. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no fungal growth around the patch The figures are shown in 45 & 46 and the results are shown in Table No: 31.

For Bacteria

After 24h the plates were observed. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no bacterial growth around the patch. The Figures are shown in 47&48 and 49&50 results are in Table No: 30.

FUNGI

Fig No. 45: ***Aspergillus niger***



FigNo.46: ***Candida albicans***

GRAM POSITIVE BACTERIA

Fig No.47:*Bacillus subtilis*

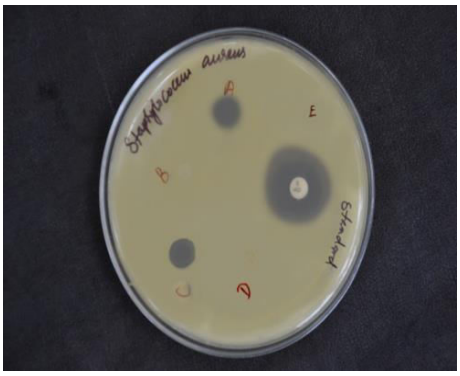


Fig No.48:*Staphylococcus aureus*

GRAM NEGATIVE BACTERIA

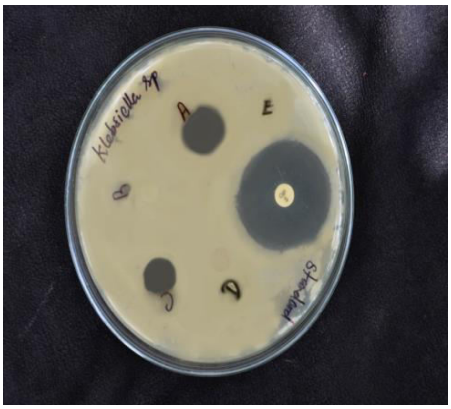


Fig No. 49: *Klebsiella pneumonia*

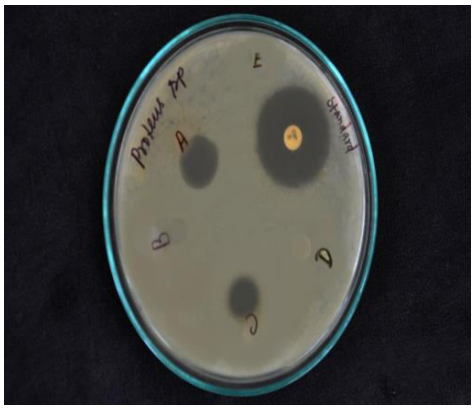


Fig No. 50: *Proteus vulgaries*

Table No: 30. Screening of Antimicrobial activity

S.No.	Name of the microorganism	A	B	C	D	E
1.	*Aspergilus Bniger* (NCIM 105)	15	-	14	-	35
2.	*Candida albicans* (NCIM 3102)	20	-	15	-	32
3.	*Bacillus subtilis* (NCIM 2063)	17	-	17	-	40
4.	*Staphylococcus aureus* (NCIM 2079)	15	-	15	-	35
5.	*Klebsiella pneumonia* (NCIM 2098)	18	-	16	-	30
6.	*Proteus vulgaries* (NCIM 2027)	21	-	15	-	30

A: Pectin (P2); B: pectin without drug patch ; C: Sodium alginate (S5) ; D: sodium alginate without drug & Standard :(Ciprofloxacin 5µg/ disc for bacteria; Nystatin 100 µg/ disc for fungi)

When compared to S5, P2 showed greater inhibition against *Candida albicans*, *Klebsiella pneumonia* and *Proteus vulgaries.* So P2 has been selected for *ex vivo* and stability studies.

7.13. *Ex vivo* study

Fig No. 51 :Goat abdomen skin tied on open ended cylinder

Table No: 31. *Ex vivo* Transdermal Permeation of P2

S. No.	Time in min	% Drug diffusion
1	0	0
2	30	0.30±0.15
3	60	0.43±0.53
4	90	1.35 ±0.71
5	120	2.23±0.62
6	150	3.12±0.45
7	180	6.45±0.47
8	210	11.28±0.16
9	240	20.45±0.32
10	270	27.70±0.06
11	300	33.10±0.83
12	330	37.56±0.71
13	360	40.24±0.71
14	390	45.26±0.63
15	420	53.43±0.68
16	450	58.34±0.24
17	480	61.01±0.63

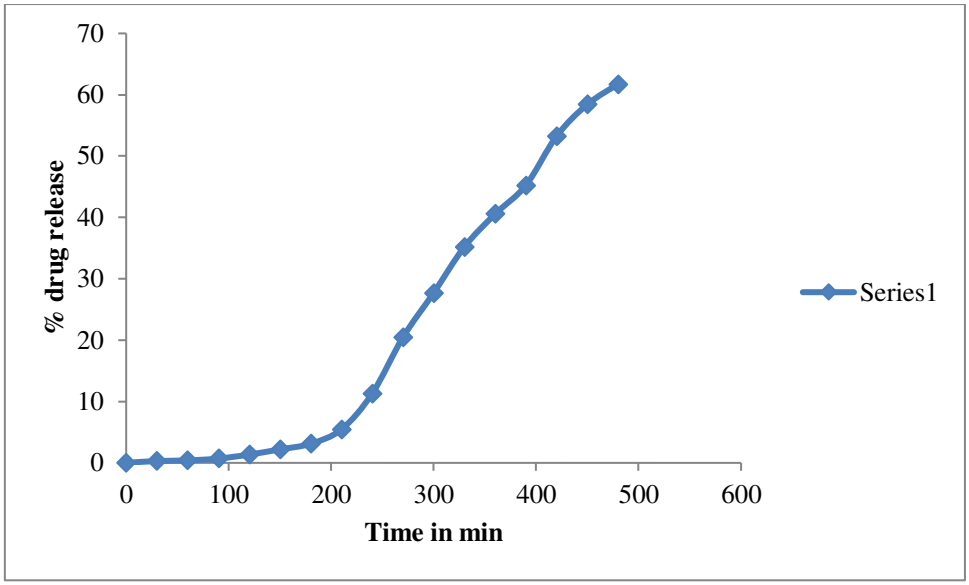


Fig No. 52: *Ex vivo* drug permeation of P2

7.14. *Ex vivo* Diffusion Kinetics

Table No: 32. *Ex vivo* release kinetic values

Formulation code	Correlation coefficient(r^2)				'n'- Diffusion Exponent
	Zero order	First order	Higuchi	Korsmeyer peppas	
***Ex-vivo* (P2)**	0.907	0.845	0.720	0.945	0.915

***Ex vivo* release kinetics**

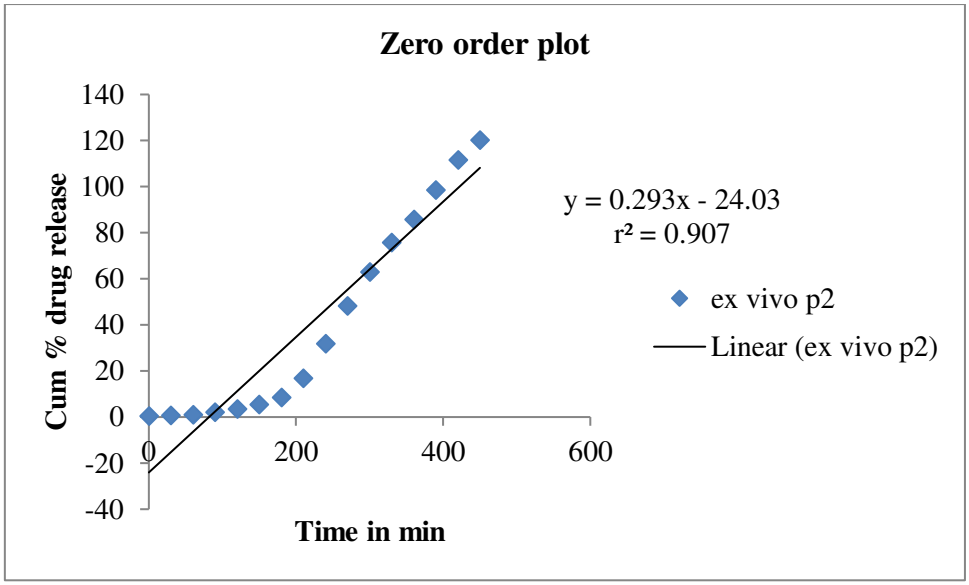


Fig No.53: Zero order plot of *ex vivo* drug diffusion of P2

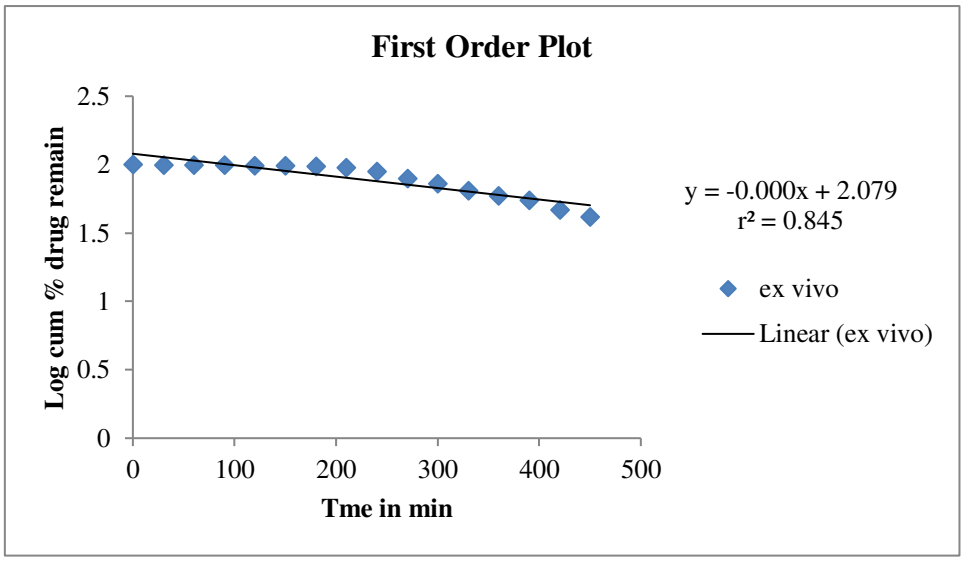


Fig No.54: First order plot of *ex vivo* drug diffusion of P2

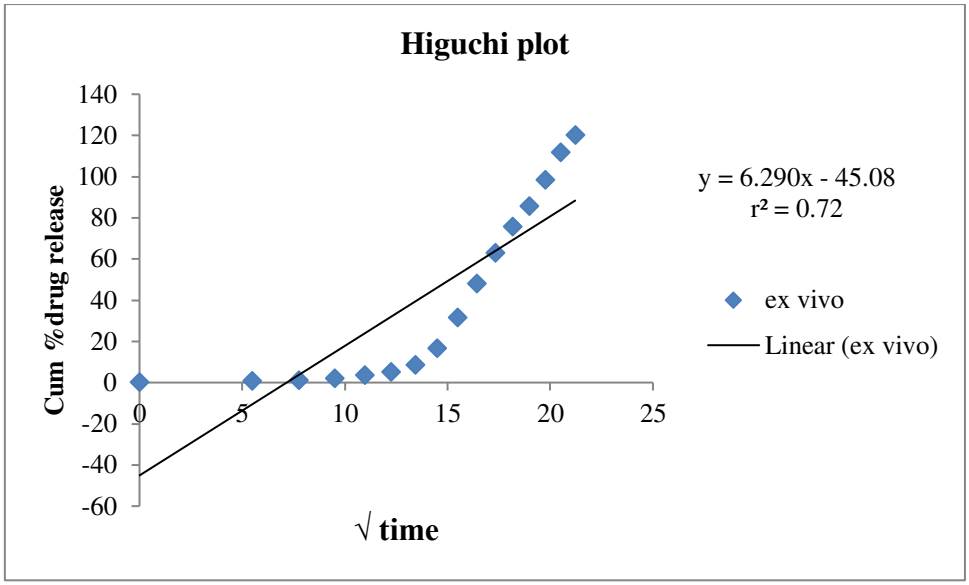


Fig No.55: Higuchi plot of *ex-vivo* drug release of P2

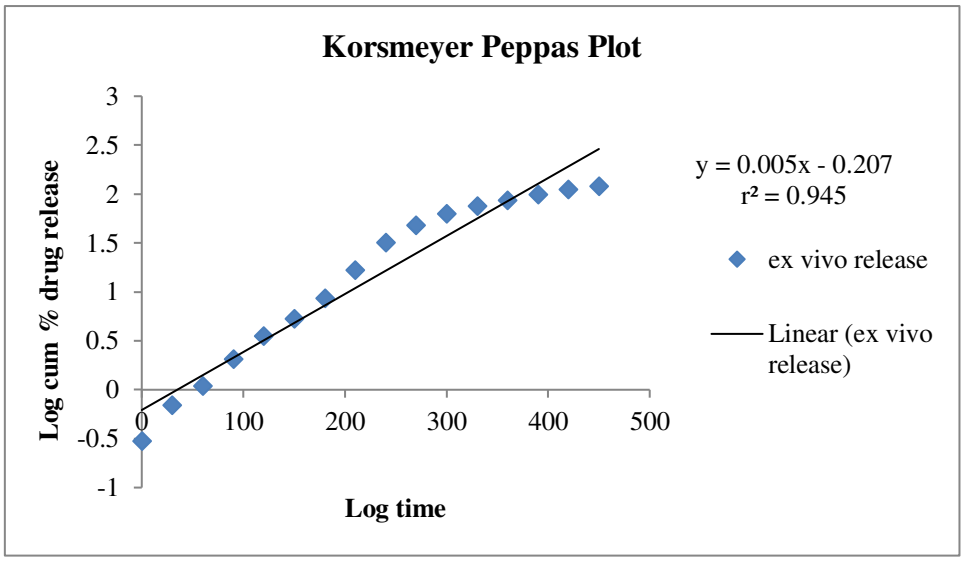


Fig No.56: Korsmeyer peppas plot of *ex vivo* diffusion of P2

Ex vivo drug diffusion kinetics of P2 shows that it follows zero order and non fickian diffusion model

7.15. Stability study of Aqueous Extract of *Azadirachta indica* A Juss. Transdermal Patch

Table No: 33. Stability study of P2

Parameter	**Room temperature**	**40±2^{0}C& RH 70±5%**
Visual Appearance	Brown colour	Brown colour
Initial	No change	No change
At the end of 1st month	No change	No change
At the end of 2nd month	No change	No change
At the end of 3rd month	No change	No change
Colour	Brown	Brown
Initial	No change	No change
At the end of 1st month	No change	No change
At the end of 2nd month	No change	No change
At the end of 3rd month	No change	No change
Texture	Smooth	Smooth
Initial	No change	No change
At the end of 1st month	No change	No change
At the end of 2nd month	No change	No change
At the end of 3rd month	No change	No change
Drug content	No change	No change
Initial	**85.69%**	**85..69%**
At the end of 1st month	85.22%	85.14%
At the end of 2nd month	84.95%	84.81%
sAt the end of 3rd month	84.21%	84.06%

The stability studies of formulation of aqueous extract of *Azadirachta indica* A Juss. transdermal patch was carried out for 3 months as per the procedure described in the table .No:5. During this period, the formulations were stable and showed no significant changes in visual appearance, colour, texture and drug content.

8.1. SUMMARY AND CONCLUSION

Six batches of (P1, P2, P3, S4, S5 & S6) Aqueous extract of *Azadirachta indica* transdermal patches were prepared by solvent casting technique.

The various formulation parameters, Drug-Polymer ratios and permeation enhancers were optimized to get thin, transparent, smooth, stable and high permeable transdermal patches.

The FTIR graphs of drug, excipients and formulations showed that there is no extra peak (or) broadening of peaks were observed and thus it indicates that there is no incompatibility between drug and excipients.

From the optimization, best 2 formulations P2 & S5 were selected based on physico chemical evaluation and *in vitro* drug diffusion study

0.3ml of glycerin was added as plasticizer to produce a flexible patch without having major influence on their diffusion property. If the amount exceeds, the film loses its flexibility and become stiff.

The plasticizer diffuses through the patch and softens the polymer particles. This softening promotes latex coalescence and patch formation.

All the six batches were evaluated for Percentage Moisture uptake, Percentage Moisture content, Thickness, Folding Endurance, Percentage Drug content, Percent Elongation, Tensile strength and Adhesive strength.

The formulations P2 & S5 showed maximum % Moisture uptake, Moisture content, Thickness, folding endurance, % Drug content, Percent elongation, Tensile strength

No significant difference in drug content was observed between the patches among the six formulations. This indicates the homogenous dispensing of drug during the patch preparation.

The data obtained from *in vitro* diffusion profile of selected formulations were fitted with various kinetic equations to determine the mechanism of drug diffusion and diffusion rate as indicated by higher correlation coefficients (r^2). The drug diffusion from P2 & S5 follows zero order and non-fickian diffusion.

These findings indicates that the drug diffusion from the P2 & S5 patch was diffusion controlled.

From the results of *in vitro* diffusion and physicochemical studies, P2 & S5 was concluded as best formulation. Then they were subjected to screening of anti microbial activity

The anti microbial screening result showed that the P2 was highly inhibit the microbial growth around the patch. So, P2 was selected for further evaluations such as *ex vivo* and stability studies.

The *ex vivo* studies results showed 61.01% of drug diffusion at the end of 8 h. It concluded the controlled diffusion property of through the skin

. The stability studies results showed that there is no significant change from its initial nature till the period of three months at 40˚C ±2˚C/75 5% RH.

The present work has achieved the objectives of formulation of transdermal patch of Aqueous extract of *Azadirachta indica* by using different polymers. The diffusion kinetics confirms that the formulation followed zero order, non-fickian diffusion model.

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